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# COMPARATIVE HISTOCHEMICAL STUDIES ON CESTODES

## I. THE DISTRIBUTION OF GLYCOGEN IN *HYMENOLEPIS DIMINUTA* AND *RAILLIETINA CESTICILLUS**

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Since numerous physiological studies with endoparasitic organisms have shown considerable differences, often between taxonomically closely related forms, a comparative histochemical study was conducted on 2 cyclophyllidean cestodes to determine what differences, if any, exist in the distribution of certain substances in their bodies and, if possible, to determine the significance of these substances in the metabolism of the tapeworms. *Hymenolepis diminuta* (Rudolphi, 1819) and *Raillietina cesticillus* (Molin, 1858) were particularly well-suited for this purpose since they were readily available and since concurrent studies on their metabolism were being made in the same laboratory.

### MATERIALS AND METHODS

*Hymenolepis diminuta* and *Raillietina cesticillus* were raised in the laboratory in white rats and chickens respectively. The adult male Sprague-Dawley rats used for *Hymenolepis* infections were given 2 or 10 cysticercoids, 2 to 3 months previous to their sacrifice for experiments. Generally, 2 worms were recovered from 2-cysticercoid infections, but the number recovered from 10-cysticercoid infections varied. The cysticercoids were raised in adult *Tenebrio molitor* bred in the laboratory. The rats were maintained on a diet of "Purina Laboratory Chow" and weighed between 300 and 500 grams at sacrifice.

The laboratory hosts for *Raillietina* were White Rock chickens of mixed sexes, 8 days of age at infection. They were maintained on a diet of standard commercial growing mash. Each chicken was given 10 cysticercoids of *Raillietina cesticillus,* which had been reared in laboratory-bred *Tribolium* sp. The number of worms recovered after 19 to 37 days of infection varied from 2 to 9. Only normal-appearing mature worms which showed evidence of apolysis were used in this study.

The worms were fixed within 5 to 10 minutes after removal from the gut by laying them outstretched on a strip of filter paper, and applying hot picro-formalin-alcohol to them with a medicine dropper. Then they were removed to a Petri dish containing the fixative solution. The tissues were dehydrated in alcohol, cleared in chloroform, and embedded in paraffin. Sections were stained by the McManus (1946) periodic acid-Schiff method (PAS), using the Schiff reagent of de Tomasi (1936) and covering the sections with celloidin before hydration. Control sections were digested in several changes of saliva for ½ to 1 hour at room-temperature, dehydrated, and then carried through the staining procedure along with the non-extracted sections. Some preliminary studies were done using the Bauer-Feulgen method (after Bensley, 1939).

### RESULTS

*Hymenolepis diminuta*

The distribution and relative amounts of glycogen appear to be identical in *Hy-*

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*menolepis diminuta* from 10-worm infections and from 2-worm infections. Most of the glycogen in the scolex is concentrated in the anterior network of osmoregulatory canals, although a small amount is scattered throughout this organ. Plate I, figure 1, shows the heavy deposits of glycogen which occur in the sucker-cups, especially at the base of the radial musculature. At the posterior end of the scolex, heavy concentrations occur adjacent to the inner walls of the 2 larger ("ventral") osmoregulatory canals.

In the region of strobilization preceding the differentiation of the reproductive systems, glycogen occurs in heavy deposits in the medullary region (i. e. the region central to the ring of parenchymal musculature), especially adjacent to the osmoregulatory canals, and as scattered deposits in the cortical region (Plate I, fig. 2). Although it is difficult to ascertain, it appears that most of the glycogen storage is intracellular, at least in the medullary region. The size of the glycogen globules decreases toward the periphery, the subcuticular layer containing only a small amount of glycogen in the form of small scattered droplets. However, throughout the parenchymal regions, small deposits also occur between the larger ones. The parenchymal muscles can not be distinguished because of the heavy glycogen deposits in the adjacent tissue. However, these contain small amounts of glycogen since they give a distinct positive reaction in worms removed from starved hosts, in which glycogen had been lost from the adjacent tissue. The subcuticular muscle layer, the cuticle, and the walls of the osmoregulatory canals appear to be entirely free of glycogen. This is the general pattern of glycogen distribution throughout the tapeworm body and it is modified only by the inclusion of various organs in more advanced proglottids.

In the region of differentiation of the reproductive systems, and in the mature region (Plate I, fig. 3) glycogen does not occur within the ovary or testes, although strands of parenchyma between the lobules of the ovary contain small deposits and the parenchyma adjacent to the ovary contains large amounts. In the early stages of development, the vitellarium is completely free of glycogen, but in successively more advanced stages increasingly more glycogen is present in diffuse granular deposits. These deposits are greatest when this organ and the entire proglottid appear mature. In the mature proglottids a structure, thought to be Mehlis' gland, located just dorsal to the vitellarium and consisting of a group of cells clustered about the ootype, contains a PAS-positive material, not extractable with saliva. This is diffusely distributed within the cytoplasm of the cells, with especially heavy concentrations occurring adjacent to the lumen of the ootype. The walls of the cirrus pouch, cirrus, ejaculatory duct, internal seminal vesicle, and vagina appear to contain no glycogen, while those of the sperm duct, vasa efferentia, external seminal vesicle, seminal receptacle, and seminal canal contain small amounts. The walls of the oviduct are glycogen-free, but the limiting membranes of the vitellarium, ovary, and testes contain large amounts as do the sperm in both the seminal receptacle and seminal vesicles. In ripening proglottids also, the sperm stain heavily; only in the terminal stages of degeneration do they give a negative reaction. In late mature proglottids, a few sperm within the testes give a positive reaction. This does not occur in younger proglottids.

In ripe proglottids the parenchyma still contains large amounts of glycogen (Plate I, fig. 4). The ova, while still without shells in the developing uterus, con-

tain small amounts of glycogen; more posteriorly, in the fully-ripe proglottids, a heavy layer of glycogen surrounds the embryos. In the shelled eggs this layer is situated between the embryo and the embryophore. Also, the area between the embryophore and the egg-shell contains small amounts of glycogen in a diffuse state and a polysaccharide not extractable with saliva.

*Raillietina cesticillus*

Generally, the distribution of glycogen in *Raillietina cesticillus* is similar to that in *Hymenolepis*. The general pattern of glycogen distribution in the entire worm can be seen in the region of early strobilization (Plate II, fig. 1). The area from the cuticle to the base of the subcuticular cells contains less glycogen than any other part of the worm, excluding certain organs, although here there is a diffuse distribution of small droplets of glycogen. Central to this is an area with heavy deposits of large globules. This zone is centered on the bands of parenchymal musculature, although these can not be distinguished. The area just inside this region contains a diffuse distribution of smaller globules of glycogen, the total amount being less than in adjacent regions but considerably more than in the subcuticular region. Lastly, there is a heavy concentration of large globules of glycogen in the center of the medullary region adjacent to and between the osmoregulatory canals. In the presence of the reproductive systems, there are heavy deposits adjacent to the various organs. The subcuticular muscles can not be distinguished with certainty except in the control sections where it is evident that these, as well as the parenchymal muscles and certain areas in the center of the medullary parenchyma, contain a diffuse distribution of a polysaccharide not extractable with saliva. No glycogen can be demonstrated in the walls of the osmoregulatory system or in the cuticle. In *Raillietina*, as in *Hymenolepis*, the bulk of the glycogen storage appears to be intracellular.

In the mature region of *Raillietina* (Plate II, fig. 2), as in *Hymenolepis*, glycogen does not occur within the ovary and testes. The vitellarium, when first evident, does not contain an appreciable amount of glycogen, but in more mature proglottids, glycogen is present in a diffuse granular state within the cytoplasm of the cells. Mehlis' gland, as in *Hymenolepis*, stains a bright pink, but the color reaction is present also in saliva-extracted controls. The positive material has the same location within the cells as described for *Hymenolepis*. The walls of the vasa efferentia, sperm duct, seminal vesicle, vagina, seminal receptacle, and seminal canal do not contain glycogen, although the sperm within these stain heavily. The ejaculatory duct, cirrus, and cirrus sac stain a light pink, but the positive reaction also occurs in saliva-extracted sections. The walls of the oviduct do not stain except at the position of Mehlis' gland. In ripening proglottids, but not in younger proglottids, some of the sperm within the testes stain for glycogen. The sperm within the seminal receptacle and seminal vesicle give positive reactions even in nearly gravid proglottids, and in gravid proglottids the sperm are still PAS-positive, although disintegrated and recognizable only by reference to preceding sections.

When the embryos first appear in the uterus of ripening proglottids, a small amount of glycogen is demonstrable in the yolk cells surrounding them. In more gravid proglottids (Plate II, fig. 3), the embryos had gained in glycogen content. In the shelled eggs a heavy layer of glycogen surrounds the embryo and the embryo-

phore gives a positive reaction for this substance whereas it does not in *Hymenolepis*. Occasionally, large globules of glycogen occur within the parenchymatous pouches, as well as within the egg. A small amount of non-glycogenous material occurs within the embryo itself.

Although no quantitative measurements were made, it appears that *Raillietina* stores more glycogen per unit cross-sectional area than *Hymenolepis*. This is apparent when one compares figures 2, 3, and 4 of Plate I with figures 1, 2, and 3 of Plate II, although the difference is exaggerated by different magnifications and exposure times.

## DISCUSSION

Using various histochemical techniques (iodine, Best's carmine, Bauer-Feulgen, and various modifications of the periodic acid-Schiff method), glycogen distribution has been studied in a number of cestodes by Brault and Loeper (1904 a, b), Busch 1905 a, b), Ortner-Schönbach (1913), von Brand (1933), Wardle (1937), Smyth (1947, 1949), and Yamao (1952). The most extensive study was that of Ortner-Schönbach on *Anoplocephala plicata, A. perfoliata, A. mamillana, Calliobothrium coronatum,* and *Caryophyllaeus mutabilis,* while the cestode most studied was *Moniezia expansa* (von Brand, 1933; Wardle, 1937; Yamao, 1952).

The general pattern of glycogen distribution is similar in all these species. The cuticle is glycogen-free and the subcuticular tissue contains little or none. Most of the glycogen storage is in the parenchymatous tissue, although, except for considerable amounts in the suckers of *Anoplocephala* spp. and the bothria of *Calliobothrium,* there is very little in the scolex parenchyma. The muscle-fibers are glycogen-free but difficult to distinguish because they are surrounded by tissue rich in glycogen. The nervous, excretory, and reproductive systems are almost free from glycogen, although the glycogen content of the vitelline glands varies from small amounts in *Caryophyllaeus* to none in *Moniezia*. The sperm generally give a positive reaction, while the reaction of the ova varies. In *Anoplocephala* spp. a small amount is present in the uterine eggs, while in *Moniezia* the uterine eggs contain none. Generally, there is no glycogen in the ovary.

Smyth (1949) found glycogen distributed primarily in the parenchymal area of the plerocercoid larva of *Ligula intestinalis,* although the intercellular spaces of both circular and longitudinal muscle layers contained large amounts. Small globules were present in the small lumina of the longitudinal muscles as seen in transverse section, and the subcuticular spaces were also filled with glycogen. In a plerocercoid of a diphyllobothriid cestode, glycogen was demonstrated primarily in the intercellular spaces of the parenchyma and the outer periphery of the longitudinal muscles (Smyth, 1947).

The distribution of glycogen in *Raillietina cesticillus* and *Hymenolepis diminuta* agrees generally with histochemical studies of glycogen in the other cestodes discussed above. Both of these worms contain moderate amounts of glycogen in the vitellarium and large amounts in the eggs. In these respects, considerable variation has been observed among cestodes. The main difference between the two species studied here appears to be in the non-glycogenous polysaccharides they contain. *Raillietina,* in contrast to *Hymenolepis,* contains considerable amounts of non-glycogenous material in the parenchymal and subcuticular musculature, the medullary parenchyma, and the walls of the male and female sperm tracts. Both contain PAS-positive material in the cells of Mehlis' gland. Smyth (1956) reports that Mehlis'

gland in *Schistocephalus solidus* also contains a PAS-positive material negative to reactions for glycogen.

The most important storage place for glycogen in both worms is the medullary parenchyma. Organs other than the vitellarium contain no demonstrable polysaccharide. Thus it seems clear that the parenchymal stores of glycogen probably serve as reserve food materials that can readily be drawn upon by various organs. The heaviest deposition of glycogen occurs adjacent to the reproductive organs, especially the ovary which in both cestodes is multilobular in shape, possibly as an adaptation to facilitate the mobilization of nutrient substances from the parenchyma. Although the studies of Smyth (1954, 1956) have shown that a major function of the vitelline glands in certain cestodes and trematodes is to provide certain proteins for egg-shell formation and the studies of others have failed to demonstrate glycogen in the vitelline glands of certain cestodes, it appears from this study that at least a part of the glycogen present in the embryos of *Hymenolepis* and *Raillietina* is provided by vitelline cells. In both worms glycogen is evidently wasted, since the terminal gravid proglottids contain considerable amounts of glycogen which is evidently not available to the embryos.

The sperm within the seminal receptacle and seminal vesicle contain large amounts of glycogen, and their glycogen content is not diminished significantly in nearly gravid proglottids. These glycogen stores may be correlated with the strenuous flagellar activity probably necessary to travel from the testis to the oviduct, whether of the same proglottid, adjacent proglottids, or nearby worms. However, since sperm within the seminal receptacle appear not to contain significantly less glycogen than those in the seminal vesicle, it is thought that the sperm may contribute significantly to the glycogen stores of the fertilized ovum. A low metabolic rate in the sperm within the seminal vesicle and the seminal receptacle, due perhaps to an accumulation of expired carbon dioxide in the medium, would explain the persistence of the glycogen stores in degenerating sperm in nearly gravid proglottids.

## SUMMARY

A comparative histochemical study of the distribution of glycogen in *Hymenolepis diminuta* and *Raillietina cesticullus* was made. Using the periodic acid-Schiff method and saliva-digested controls, it was demonstrated that both cestodes store large amounts of glycogen in the parenchyma, especially in the medullary region adjacent to the osmoregulatory canals and the reproductive organs. The mature sperm also give a positive reaction. Except for the vitellarium, which contains glycogen when mature, the reproductive organs are entirely free of polysaccharide. The significance of the distribution of glycogen in these cestodes is discussed and literature pertaining to glycogen distribution in cestodes is reviewed.

## ACKNOWLEDGEMENTS

Acknowledgements are due to Dr. Asa C. Chandler for his aid in obtaining an understanding of the morphology of *Hymenolepis diminuta* and *Raillietina cesticillus*, and to Dr. Allen C. Enders for his advice regarding histological and histochemical technique and for the use of the facilities in his laboratory. Also, we wish to thank Mrs. Evelyn Hake, who was responsible for the maintenance of *Hymenolepis diminuta* in laboratory rats, and Mr. William B. Foster, who supplied the *Raillietina cesticillus*.

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## Explanation of Plate I

Figure 1. A section through the scolex of *Hymenolepis diminuta* showing heavy deposits of glycogen at the base of the radial musculature in the suckers and small scattered deposits throughout the remainder of the scolex parenchyma, including the parenchyma of the rostellum (above the 2 suckers in the figure). Periodic acid-Schifff method. 450×.

Figure 2. Transverse section showing the distribution of glycogen in the region of strobilization of *Hymenolepis diminuta*. Dorsal and ventral osmoregulatory canals as well as a transverse canal are visible in this section. Periodic acid-Schiff method. 100×.

Figure 3. Transverse section through a mature proglottid of *Hymenolepis diminuta* showing heavy concentrations of glycogen in the parenchyma and in the sperm within the seminal receptacle and seminal vesicle (left), but none in the ovary and testes. Periodic acid-Schiff method. 35×. (Print enlarged approx. 1½×.)

Figure 4. A transverse section through a gravid proglottid of *Hymenolepis diminuta* showing the distribution of glycogen in the parenchyma and in the eggs. Periodic acid-Schiff method. 35×.

PLATE I

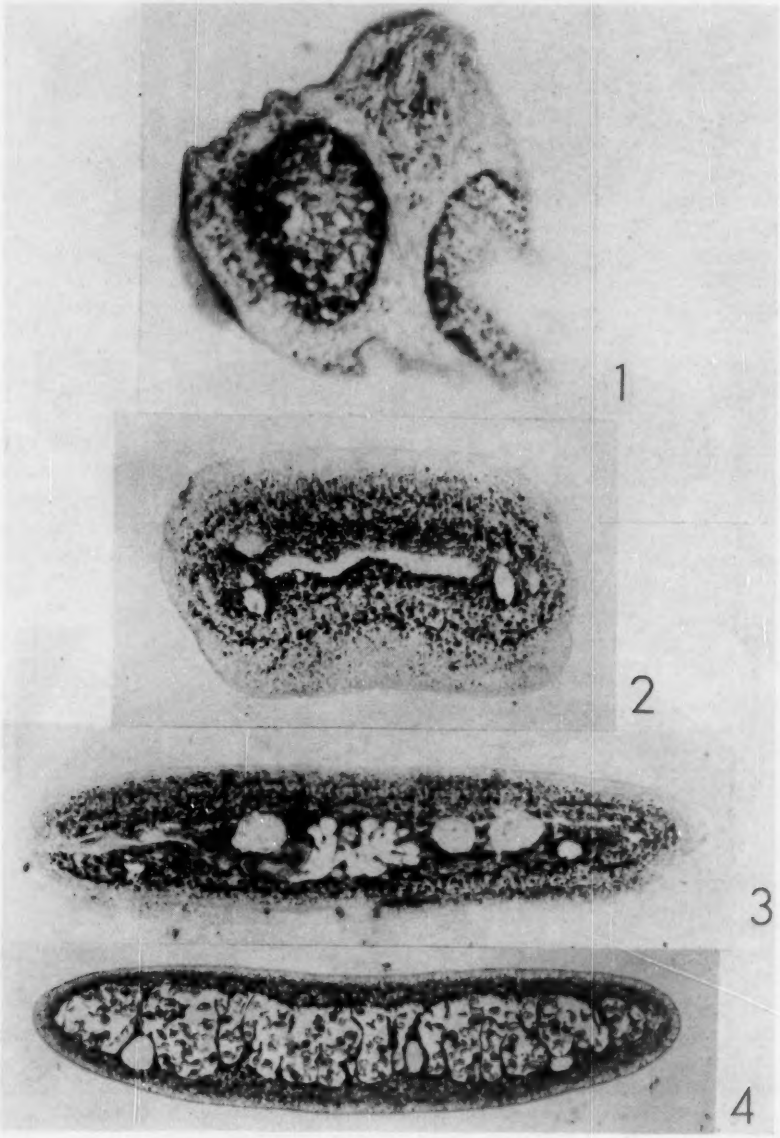

PLATE II

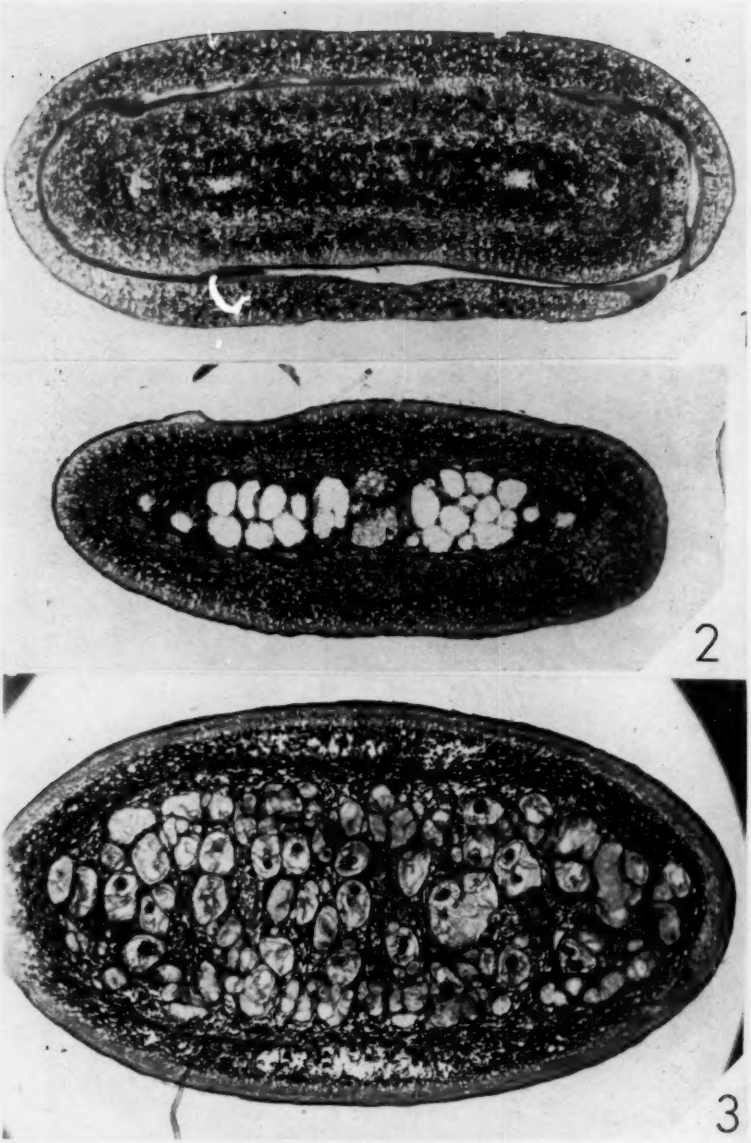


Figure 1. Transverse section showing the distribution of glycogen in the region of strobilization of *Raillietina cesticillus*. The section was made through the anterior part of 1 proglottid and the overlapping posterior border of the adjacent proglottid. Periodic acid-Schiff method. 100×.

Figure 2. A transverse section through a mature proglottid of *Raillietina cesticillus* showing heavy deposits of glycogen in the parenchyma and no deposits in organs other than the vitellarium (center). Dorsal to the vitellarium, Mehlis' gland surrounds the ootype. Periodic acid-Schiff method. 20×.

Figure 3. A transverse section through a gravid proglottid of *Raillietina cesticillus* showing heavy deposits of glycogen in the parenchyma and in the eggs. Periodic acid-Schiff method. 20×.

# MORPHOLOGY AND DEVELOPMENT OF ONCOSPHERES OF THE CESTODE *OOCHORISTICA SYMMETRICA* BAYLIS, 1927

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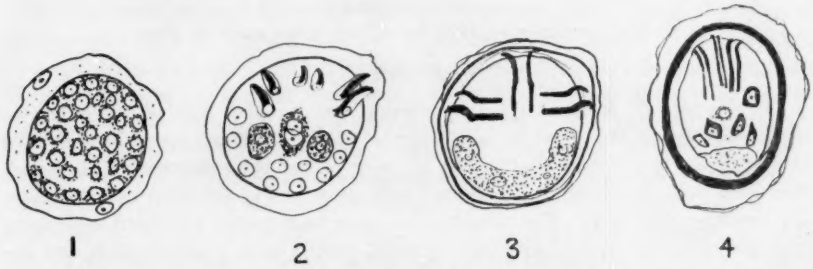
In a recent paper (Ogren, 1957) the embryogeny of this tapeworm was described. The present paper deals exclusively with the structural and developmental changes of the oncosphere although both topics were summarized in an abstract (Ogren, 1956b). It has not been generally realized that the hexacanth embryo contains differentiated structures and undifferentiated embryonic cells. Moreover, the changes by which the sphere of blastomeres becomes an hexacanth embryo have not been described. In fact, this series of changes was not realized by the writer until after publication of the development of *Mesocestoides* (Ogren, 1956a).

The oncosphere is a muscular embryo that manifests a series of cellular changes during its development before being eaten by the intermediate host. A knowledge of these changes helped in understanding how it is that oncospheres are prepared for infectivity and what original structures are present. Knowledge was also obtained of secretory activity by embryonic cells.

The oncosphere of *Oochoristica* is a large spherical, muscular embryo (Fig. 19) characterized by the possession of 6 large hooks, a body of mesenchymal cells and irregular granular epidermal areas. Each oncosphere is enclosed by an outer shell and an inner non-cellular pseudoembryophore. The purpose of this paper is to describe the development of these structures. Answers to the following questions




TEXT-FIGURE 1. Diagrammatic stages of oncosphere development.

are sought: 1) How does the inner non-cellular membrane arise? 2) When do the granular areas appear in the oncosphere? What is their function? Are they penetration glands? 3) What happens to the epidermis? Is it ectoderm? 4) How do hooks develop? 5) Do compact plastin cells appear? 6) What is the appearance of the contractile tissue? 7) What is the systematic importance of this study?

A general picture of oncosphere development was formulated consisting of 4 structural phases of development. These are artificial divisions and overlapping occurs. However, they are useful for description and investigation. The phases are in general typical of development of other oncospheres (Text-Fig. 1).

1. *Morula:* This was a solid ball of cells resulting from cleavage and showed very little differentiation.

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2. *Hook Formation, parenchyma and muscle development:* Three pairs of hooks appeared from oncoblasts and mesenchymal cells formed muscle fibers concentrated in the hook region.

3. *Membrane Formation* and *Epidermal Gland Development:* A non-cellular pseudoembryophore developed from oncosphere secretions that became a tough, thick protecting covering. Granular epidermal areas developed, spread over the oncosphere cortex and formed a secretion that appeared to give rise to membranes.

4. *Plastin Cell Formation:* Oncospheres in the oldest segments became smaller and more compact. A few granular mesenchyme cells transformed into compact plastin cells. This was apparently a preparatory period for infection.

## MATERIALS AND METHODS

Specimens for these studies were obtained from laboratory infections of *Oochoristica symmetrica* (= *O. ratti*) in white rats and white mice. All tapeworms were killed immediately in alcohol-formalin-acetic acid (AFA), Carnoy's or Regaud's fixatives. The following stain procedures were used: Methyl green-pyronin, toluidine blue, Delafield's hematoxylin, Lillie's metachromatic stain using Weigert's acid hematoxylin and safrinin O counterstain, van Gieson's connective tissue stain, aniline acid fuchsin for mitochondria and muscle, periodic acid leucofuchsin (Schiff) procedure and the ninhydrin Schiff procedure, neutral red as an *in vivo* stain. Histological methods as described in previous papers were employed. Living oncospheres were observed as described previously (Ogren, 1955a).

## OBSERVATIONS

(All figures, except when identified as text-figures are in the plate.)

### *Morula*

On completion of cleavage, the embryo was a solid ball of large blastomeres enclosed by the vitelline membrane or shell. Cells arranged in a definite layer at the surface of the embryo (Fig. 1), were packed close together. Cell boundaries were easily recognized. Medullary cells were loosely arranged with considerable space between cells. The cytoplasm of these blastomeres was filled with many large granules that stained red with pyronin, purple with toluidine blue, and appeared related to mitochondria. As the hooks began to appear, epidermal cells manifested cytoplasmic changes. The cytoplasm became more vacuolated, the borders of cells fused and the granules tended to clump around the nucleus and at the cell surfaces (Fig. 10). The epidermis did not separate from the rest of the embryo as in taeniid development, but was retained as the cortex of the oncosphere.

### *Hook-Formation, Parenchyma, Muscle Formation*

The oncoblasts were large epidermal cells (Fig. 1) with many large granules, probably representing mitochondrial bodies, since they contained ribonucleic acid and were metachromatic. Contrary to the writer's previous views (Ogren, 1956a) the hooks actually were formed inside the cell and were then forced to project outward. The blade was formed in the cell (Figs. 2–4) to one side of the nucleus and appeared as a crescent-shaped metachromatic body. It was considered likely that the cell granules either secreted the hook substance, or broke down to form the hook substance. The abundance of granules decreased as the hook developed. Onco-

blasts of mature hooks were difficult to identify for this reason. The blade continued to enlarge in the cell (Fig. 4) until the base of the blade was formed and part of the shank (Fig. 5). At this time the hook began to project through the cell surface (Fig. 6). The remainder of the shank was formed in the same cell, a fact which forced the blade to project further from the surface. The thickened collar with its barbs was formed at the margin of the cell. Fully developed hooks had the appearance shown (Figs. 7–9). They represent the typical hook shapes found in this embryo. Other hooks figured on the plate were made by a similar process. The hooks in living and fixed material were not always observed from the same angle. A slight rotation, or an oblique view resulted in slightly different proportions, or in failure of all hooks to remain visible at the level drawn. The purpose in representing the hooks was not always to illustrate their form, but to employ the hooks as a means of orientation.

The original oncoblasts may have been retained associated with the contractile fibers of the parenchyma moving the hooks. The blades did not always appear to develop at once, occasionally the lateral blades appeared first. The 6 hooks were not arranged on the same plane. If all hooks are considered to be pointing toward the ventral surface, then the lateral members of a pair were arranged one above the other (Fig. 29). Thus the dorsal-lateral hooks (Fig. 7) were more slender than the large clumsy ventral-lateral ones (Fig. 8). The blades of the lateral hooks were also more gradually curved than the slender, sharply pointed medial hooks (Fig. 9).

The body tissues of oncospheres were enclosed by a thin cuticle. Mesenchymal cells were located beneath this cuticle around the spherical body (Fig. 10). Nuclei of these cells were of different sizes and occasional mitotic figures were seen. The mesenchymal cells were irregular in shape with very little peri-nuclear cytoplasm. Their cell processes ramified between adjacent cells forming a complex matrix. The peri-nuclear cytoplasm of these cells contained many large granules that stained for RNA, were metachromatic, and were stained pink with aniline acid fuchsin stain for mitochondria. These mesenchymal cells in young oncospheres had a large distinct nucleolus. As the hooks developed, a prominently stained pair of cells appeared behind and below the medial hooks in the medullary parenchyma (Fig. 11). This primordium in older oncospheres became the center of abundant muscle fibers (Figs. 13–15). This binucleate primordium was a real mystery. It was shown to be a group of closely united cells, 2 to 4 in number. Large prominently stained RNA granules were abundant in the cytoplasm. At various times the cells were considered by the writer to be gland cells, oncoblasts, germinative cells. Careful study clearly showed (Fig. 13) that they were closely associated with small muscle cells and themselves were part of a contractile mass (Fig. 14). These observations serve to emphasize the true nature of cestode muscle as a group of long contractile fibers closely associated with many large granular myoblasts. Muscle cells from oncospheres, although smaller, were nevertheless typical of those found in the adult parenchyma. In fact the oncosphere was largely a mass of contractile mesenchymal cells. This contractile tissue was similar to that first clearly seen in the early cysticercoid and consisted of large granular cells closely associated with thick contractile fibers (Ogren, 1957). These observations provide a start toward a knowledge of muscle histogenesis in cestodes. The small and large myoblasts associate closely and their protoplasmic fibers fuse. The fiber thickens by the fusion

of adjacent fibers and the increase of contractile cytoplasm from the cell. The presence of a contractile center near the medial hook shanks was important. Definite contractile fibers, associated with the cells of the contractile center, passed to the medial hooks (Fig. 13). Occasional mitotic figures were seen here in the center. The smaller nuclei represent more recent divisions. There was also an abundance of contractile substance around the collars beneath the cuticle. A large contractile mass was attached to the collar of each lateral hook (Fig. 13). These were the largest muscle masses in this oncosphere, always stained clearly, and were visible in living specimens. The myoblasts of this muscle were also adjacent to the fibers. This oncosphere muscle had affinity for collagen stains. The parenchyma was actually a contractile stroma, especially well seen in specimens treated with the allochrome periodic acid Schiff procedure after bromination. All glycogen and other polysaccharide substances were removed by this means leaving a red-stained matrix enclosed by the cuticle.

*Membrane Development*

A thin transparent membrane comprised the outer shell or capsule of the embryo. It was derived from vitelline material early in development. This shell was united to certain nuclei, principally derived from vitelline cells and polar bodies (Figs. 1, 10). Nuclei from the uterine capsule were also closely attached in sectioned material. It is not known if these nuclei are retained. Metachromatic, pyronin granules were abundant in the space around the oncosphere (Fig. 11). These granules may have belonged to the RNA granules of disintegrated cells. In the oldest "eggs" with oncospheres this area showed the presence of many rod-like mitochondrial bodies by the use of aniline acid fuchsin (Fig. 24).

Following the appearance of the granular epidermal glands and their spreading over the surface of the oncosphere, the shell capsule pulled away from the embryo, leaving a space around the embryo. This region, especially in Carnoy-fixed specimens, contained an homogenous colloid secretion (Fig. 25) that stained pink with safranin O. The substance was essentially similar to the colloid secretion in the epidermal glands. The oncosphere also became draped by a thin membrane (Fig. 12). As the epidermal glands continued to develop and secrete, a membrane with metachromatic properties formed beneath the capsule and was very closely applied to the oncosphere (Fig. 21). In favorable cases the membrane was seen forming as a cuticle from the gland secretion (Fig. 20). In many places, especially over the gland, the cuticle of the embryo was attached to the membrane by delicate strands. This membrane represented a type of secretion whose properties were similar to cuticle and hook substance. In older oncospheres the pseudoembryophore, as an embryonic cuticle, became very thick, detached from the surface and became a separate membrane (Fig. 26). Its shape was slightly elliptical or "egg-shaped." Secretion occupied part of the new space around the oncosphere. This secretion (Fig. 23) could be seen in living preparations. Because of the intense stainability of this membrane substance, it obscured a clear view of the older hexacanth except in sections cut to 5 microns. This thick, intensely stained pseudoembryophore was present only in older tapeworms, and in the older oncospheres (Fig. 22) found in the oldest segments. The absence of such a condition may indicate an immature tapeworm strobila. It is possible to have mature infective oncospheres having very thin pseudoembryophores (Fig. 19).

The epidermal glands were still present when oncospheres entered the beetle hemocoel. Thus, after the original membranes had been removed by digestive enzymes, new protective membranes were formed by the glands or oncosphere secretions. Such new membranes were seen (Figs. 20, 32, 33) around the oncospheres only a few days in the beetle hemocoel. This was also reported by Rendtorff (1948). Chemical reactions of the pseudoembryophore indicated it was a protein gel and gave positive reactions with the xanthoproteic, Millon's reaction, Ninhydrin Schiff procedure.

*Epidermal Gland Development*

The presence of peculiar granular areas in living oncospheres quickly attracted attention (Fig. 19). The following is a description of the development of these areas. The gland area was marked out early, after the hook blades had developed and the contractile center was present, as 2 large cortical cells on either side of the center (Figs. 10, 12) containing colloidal substance. This substance was easily seen because it stained intensely blue-green with toluidine blue, red to pink with metachrome safrinin O, and blue-purple with methyl green pyronin. It was finely granular, nearly homogeneous in appearance. Large RNA granules were present at the cell periphery. The substance stained more intensely as the oncosphere became older and the cells more differentiated. Most significant was the tendency (Fig. 16) for the gland area to increase in extent. Thus more and more epidermal cells of this region became involved (Fig. 17). The result, in most cases, was a roughly C-shaped granular area (Figs. 20, 28, 32). In living oncospheres taken from young gravid segments (Fig. 19), the areas appeared as pockets of swarming active granules. The large granules colored orange with neutral red. In living oncospheres from old gravid segments (Fig. 23), there was an extensive granular area. However, the orange-stained granules were not always moving in all parts of the area, and the areas appeared as coagulated protein masses. In these older oncospheres the gland formed a granular cap over the presumptive scolex region. In free oncospheres, a viscous secretion was present (Fig. 23, 30) around the embryo. In oncospheres from the beetle, membrane secretion was found associated with the gland area (Fig. 20). These observations gave rise to the general hypothesis that one function of the secretion was the formation of membranous substance around the oncosphere.

What are the active, swarming granules? The granules stained red as mitochondria with aniline acid fuchsin procedure, but did not stain for RNA. In other living cells mitochondria are reported to show agitation and displacement related to protoplasmic currents (DeRobertis *et al* 1954, p. 155). The presence of such movement indicates a very fluid condition (low viscosity) of the cytoplasm. During differentiation of these cells, there is a lowering of viscosity. Such changes are also known to be characteristic of aging cells. The suggestion is made that the granules are related to mitochondria and represent secretion in nearly differentiated cells. Observations indicated that as the granules broke down, the secretion exuded through the cell surface to spread over the oncosphere. In a later differentiated condition the granules were much larger and there was a tendency for the cytoplasm to become more viscous, gel-like, thus preventing movement of the granules. These epidermal cells may thus properly be spoken of as unicellular glands in that they form a secretion and expell it for use of the embryo.

What is the secretion used for? The secretion was protein (probably scleroprotein) in nature and showed the same protein reactions as the pseudoembryophore. It became a viscous colloidal protein which appeared to gel, forming membranes about the oncosphere. Evidence for this was seen in the abundant viscous substance bathing the surface of the oncosphere (Fig. 30). This substance colored in the same fashion as the substance in the glands. Moreover, there were abundant membrane connections of the oncosphere with the pseudoembryophore, best seen in the gland region (Figs. 28, 33). In preparations stained by the allochrome Schiff procedure (Fig. 31), the granules were red, the inner part of the membrane was red, the outer part pink, the shell capsule blue. The same kind of stain reaction was seen with the metachrome safrinin O stain in which the gland was red, the membrane red, and the shell gray. The secretion in the glands colored green with toluidine blue and the pseudoembryophore membrane stained dark purple. This was taken to mean that the protein became modified as it formed the tough membrane. Secretion around the oncosphere was green, but substance that was transforming to membranous strands colored red purple (Fig. 28). Moreover, the surface of the gland had definite droplets of transformed secretion (Fig. 28). These colored purple, whereas the gland granules were still greenish. It is known that changes in isoelectric point can modify the metachromatic effect of toluidine blue (DeRobertis *et al* 1954). Oxidation-reduction changes may also have some effect.

Many of the above staining reactions for the pseudoembryophore and epidermal glands were those shown by the embryophore of *Mesocestoides* (Ogren, 1956a). The epidermis of the *Oochoristica* hexacanth, however, did not separate to form a cellular embryophore. The suggestion is made that the two cell groups are homologous and have a similar embryonic origin. The difference being that in *Mesocestoides* the epidermal cells separate from the oncosphere surface, whereas in *Oochoristica* they are retained as the epidermis.

The granular epidermal areas in the oncospheres of *Oochoristica* described above are obviously the "elliptical areas" first noticed by Rendtorff (1948) in oncospheres from infected beetles. They also agree with the description of the elliptical areas called "penetration glands" by Millemann (1955). The epidermal areas, however, have no ducts or pores. Their secretion passes through the body surface, a permeable non-cellular cuticle. The name *epidermal glands* is suggested as a useful nonfunctional name to replace the above terms.

The statements by Heilbrunn (1956) that metachromatic coloration with toluidine blue indicates the presence of heparin-like substances capable of preventing protoplasmic clotting, may provide a possible additional theory for penetration. Oncosphere secretions may adversely affect the cytoplasmic activity of epithelial cells, reduce intercellular adhesion by secreting anti-clotting substances and so permit active movement of oncospheres between the cells of the mucosa.

### *Plastin Cell Formation*

Development of the oncospheres continued after the formation of the epidermal glands and pseudoembryophore. The oncosphere began a period of transformation by which it became smaller and more compact (Figs. 26, 27). The pseudoembryophore also became thicker and egg-shaped. The hooks, originally projecting to the sides (Fig. 17), were pulled parallel to each other as in Figure 27. Coincident with this the cells of the oncosphere collected in the region behind the hooks. The

once granular cytoplasm around nuclei of these cells became more compact (Fig. 26) and stained very intensely with pyronin. A few cells retained a granular appearance, but most cells had some compact cytoplasm (Fig. 27). This compact cytoplasm colored intensely for mitochondria with aniline acid fuchsin after Reguad's fixation (Fig. 24). The condition was similar to that found in *Mesocestoides* and permitted calling these cells "plastin cells," or compact germinative cells. Since the oncosphere was considered to be a definitive embryonic stage, increase of plastin cells was to be sought in the early cysticercoid. Oncospheres were thus fed to starved *Tribolium confusum*. After 5 hours in the beetle, oncospheres were enclosed by a thin membrane, manifested little movement, contained 6–8 plastin cells, and had extensive granular areas with inactive granules (Fig. 32). After 7 days in the beetle, oncospheres had grown very little, were enclosed by a delicate membrane, had a somewhat reduced granular epidermal area, and contained over 20 plastin cells (Fig. 33). The epidermal areas were absent after 15 days.

### *The Living Oncosphere*

The living, unstained embryo was difficult to study. Enclosed within the vitelline shell, it was nearly obscured by the abundant clear, oily globules filling the peri-oncospheral space (Fig. 22). This condition was especially true of oncopheres from the oldest segments. Eggs were placed in mammalian Ringer's solution on a slide, then covered with a coverslip ringed with vaseline. Gentle pressure on the cover slip broke the outer vitelline shell of the egg and released the oncosphere. The oncospheres, usually still enclosed by the membranous pseudoembryophore, were immediately active. The large, clumsy hooks, continually being extended, pulled backwards, and then retracted, were the most prominent visible structures. In many oncospheres two lateral granule-filled pockets were evident. They corresponded to the granular epidermal glands of sectioned material. The granules in younger oncospheres (Fig. 19) were in violent swarming motion and resembled a bacterial culture. The granules were large, and location and movement resembled granules found in epidermal regions of oncospheres from *Hymenolepis* and *Choanotaenia*. Oncospheres stained with neutral red *in vivo* showed a regional coloration. The germinative cells (granular and compact) stained bright red or with red granules. The granules of the epidermal glands colored yellow. These granules were not always active and in certain living oncospheres appeared to fuse forming irregular masses or sheets beneath the cuticle. In oncospheres with as many as 8 plastin cells, the yellow granules of the glands covered the greatest area. The yellow granules were not very active, however, and formed a cap over this part of the oncosphere. Flame cells were not present in these oncospheres, but appeared in the late cysticercoid in the beetle (Ogren, 1957). Movements were observed similar to those of *Mesocestoides*. The loss of viability was indicated by slowing and eventual cessation of movements.

## DISCUSSION

It is now evident that among the Cyclophyllidea there is a group of tapeworms in which the embryonic epidermis is retained around the oncosphere; hence, all embryonic cells are present in the metacestode. This is in marked contrast to the group exemplified by *Mesocestoides* (Ogren, 1956a) and *Taenia* (Silverman and Maneely, 1955), in which a well developed embryophore forms from epidermal cells and separates from the oncosphere. Only mesenchymal cells forming the oncosphere become

the metacestode. The condition in *Oochoristica* is similar to development in the hymenolepid group, of which *Hymenolepis* (Ogren, 1955a) and *Choanotaenia* (Ogren, 1955b) are examples. In this group, the epidermis is retained as the surface of the oncosphere and granular epidermal glands develop as an unsuccessful attempt to form an embryophore. Thus, there are 2 basic kinds of tapeworm embryos: 1) Those in which the epidermis is retained as the cortex of the oncosphere; 2) Those in which the epidermis separates from the inner cell mass or oncosphere and becomes the embryophore, or ciliaphore, whichever the case may be.

These observations have considerable importance in relation to germ layer homologies. A review of this literature may be found in Wardle and McLoed (1952). At that time only taeniid and pseudophyllidean types of embryonic development were known. The theory was that the embryophore represented a detached ectoderm. However, since it was cast off following hatching of the oncosphere in the host intestine, the conclusion was made that the cestode had by this means lost its embryonic ectoderm. The surface of the adult tapeworm could not, therefore, be ectoderm. By this means, the absence of a cuboidal epithelium in cestodes could easily be explained. However, the theory is no longer satisfactory since there is a group of tapeworms whose "skin" is typical for cestodes, but whose embryos do not lose their epidermis ("ectoderm"). Perhaps this indicates that the epidermis of the morula and oncosphere should not be considered a true ectoderm, or that some other embryonic change is responsible for the absence of ectodermal derivatives in adult cestodes. Techniques at present do not permit settlement of this question. However, there are 2 possible views: 1) These surface cells are not potentially ectoderm. It has been learned that surface cells in invertebrate embryos may not always form ectodermal structures, but may move inwards to form mesoderm or endoderm. The position of the cells does not necessarily define their homology; rather, the final derivatives indicate this. 2) The epidermis around cestode embryos is actually an embryonic ectoderm that becomes differentiated early. The cells become old cells, fill with secretory granules and, unable to divide further, are lost, unable to take part in further differentiation. These epidermal cells do, however, form hooks, glands, cilia, and membranes, all epidermal structures. Therefore, in the taeniid cestodes the ectoderm is lost because it differentiated early into the embryophore; in hymenolepid cestodes and *Oochoristica* the ectoderm is lost because, while retained as the covering of the oncosphere, it differentiates early as secretory, hook-forming and subcuticular cells. Examination of ciliated epidermis (ciliaphore) of the coracidium, indicates that this suggestion has theoretical usefulness. This layer is similar to the embryophore and its modifications. According to Vogel, (1929, 1930) it is first a cuboidal epithelium which later differentiates, losing cellular borders and developing cilia. This ciliated covering is lost during ingestion by the copepod and does not form adult structures. These cells, even if carried into the host, would not be expected to divide further since they had already differentiated and aged in the formation of the ciliaphore. This second hypothesis can be used to explain the absence of epidermal derivatives in all groups of cestodes.

We know very little at present concerning the secretions of the embryonic cells in the oncosphere. Such activity is manifested by the formation of hooks and membranes. An additional secretion may be associated with penetration of the host intestinal mucosa. However, an enzyme has not been clearly demonstrated. 1) A

cytolytic type secretion has been suggested and has been supported by the work of Silverman and Maneely (1955) on *Taenia*. 2) An anti-clotting metachromatic substance like heparin may be present that inhibits cytoplasmic secretions and loosens cell adhesions, allowing the oncosphere to work between the cells, then enter the blood stream or hemocoel. 3) An adhesive (or clot-forming) secretion was suggested by Reid (1948) which may well be a combined property of all oncosphere secretions. These suggestions all indicate how much more we need to know in order to develop a suitable theory of penetration.

The above study of development of one oncosphere indicates that the morphology of an oncosphere is constant or fixed only when its development in the gravid segment ceases. Its further differentiation into metacestode structures indicates that potential development is greatest at this time. The comparative and developmental approach has proved to be useful in explaining the structure of oncospheres and will surely assist in our search for physiological information. For proper controls it is necessary to know what particular developmental stage is being used or is best to use for experimental studies. This appears true for studies of penetration, movement, hooks, membranes, glands, metabolism.

The histology of the oncosphere is relatively simple. Many of the differences between cells are related to the degree of differentiation, cell age, secretory activity and position in the oncosphere. The following cell types were recognizable: epidermal cells, muscular-parenchymal cells, granular and compact germinative cells (plastin cells), hook-forming cells (oncoblasts), epidermal gland cells. There remains a great deal to be learned concerning the cytology of this tiny embryo so important for cestode physiology.

Sufficient information is now available to present certain generalizations and to review briefly the relationship of development and morphology of hexacanth embryos to developmental patterns among Cestoda. Such studies may have considerable importance for cestode systematics.

Embryology has not greatly influenced cestode classification largely because so little has been known about it. Moreover, early investigators did not discover a convenient correlation, possibly because of the inadequate status of cestode classification. Studies by Moniez (1881) and Schauinsland (1886) on the embryogeny of pseudophyllidean cestodes and detailed knowledge of the coracidium, contributed by Vogel (1929, 1930), Michajlow (1933), and Vergeer (1936) confirmed a pseudophyllidean pattern. Studies of taeniid embryogeny by Janicki (1906, 1907), Spätlich (1925), Saint-Remy (1900a, 1900b, 1901), Stunkard (1940), Joyeux and Baer (1945), Venard (1938), Ogren (1956a) indicated a cyclophyllidean pattern. Harmonization of these reports into 2 patterns among cestoda was done by Fuhrman (1931), Hyman (1951) and Wardle and McLeod (1952) and reemphasized recently by Clarke (1954). However, early studies by the writer (Ogren, 1953) indicated there were at least 2 groups among the Cyclophyllidea. Reid's (1948) observation of "penetration glands" in certain oncospheres demonstrated that among Cyclophyllidea there was more than 1 kind of oncosphere morphology. Studies demonstrated that this difference was the result of a definite type of cyclophyllidean development (hymenolepid development) characterized by the absence of a separated embryophore and the presence of epidermal glands in the oncosphere (Ogren, 1955a, 1955b, 1957). Completed studies of embryogeny of *Anoplocephala* (Saint-Remy, 1900b) and *Bertiella* (Stunkard, 1940) and of *Paranoplocephala* (Ogren,

1953) suggested an additional type of embryo enclosed by the "pyriform apparatus", the anoplocephalid type. Study of development and structures of *Oochoristica* embryos (Ogren, 1957) demonstrated the presence of an additional cyclophyllidean pattern, the linstowiid type. Therefore, there are at least 7 basic embryonic groups: the taeniid, the mesocestoid, the hymenolepid, the anoplocephalid, the linstowiid, the proteocephalid, the diphyllobothriid. There is now need to study the embryogeny and oncosphere morphology of the various cestode genera to discover to which group they belong. Much important information has been omitted in the past because of failure to know what to look for. The following approach and generalizations have been successfully employed by the writer.

Four basic types of information are needed for proper comparisons (Ogren, 1956a): 1) Oocyte morphology; 2) Early cleavage pattern; 3) Embryo development and morphology, hooks and fate of epidermal cells; 4) Metacestode morphology. It is suggested that future studies of new species include all 4 points if possible, or at least numbers 1 and 3.

### *Oocyte Morphology*

Mature oocytes in the ovary or expelled into the uterus may or may not contain large vitellin particles in their cytoplasm. Studies so far indicate that cyclophyllidean oocytes tend to have several large masses; hence they have been called for convenience, *conglomerate oocytes.* Pseudophyllidea and proteocephalids examined appear to have fine granular vitelline material in the cytoplasm of their small oocytes. For convenience these have been called *granular oocytes.* This point needs careful examination since very little recent histological information exists and it appears that the cleavage pattern is related to the nature of the oocyte.

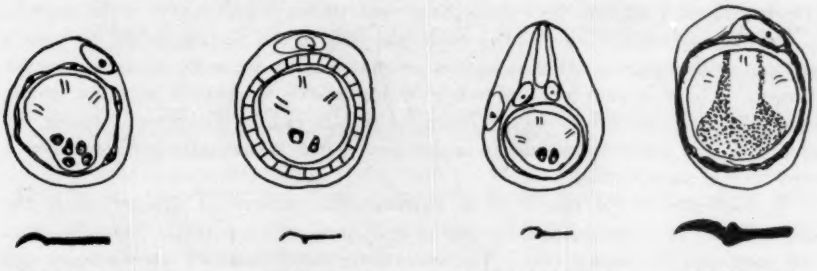
### *Early Cleavage Pattern*

It is necessary to observe the meiotic divisions and polar body-formation to interpret properly the cleavage stages of tapeworms. Thus, the first divisions of the oocyte following sperm entrance are those in which polar bodies are formed (Janicki, 1906, 1907; Richards, 1909; Motomura, 1929; Jones 1945; Douglas, 1956.) These products of meiosis have been observed in only a few cases. Moreover, the presence of large amounts of vitelline material tends to prevent participation of all blastomeres in the formation of the embryo. The hypothesis is presented that large quantities of vitelline substance present in the oocyte, retard divisions of 1 series of blastomeres, resulting in their exclusion from the embryo. Thus, conglomerate oocytes have cleavage in which 1 macromere is excluded from the morula. Granular oocytes appear to have cleavage in which all blastomeres become the embryo (Ogren, 1957). The linstowiid, the proteocephalid, and diphyllobothriid groups follow this pattern. Motomura (1929) made careful studies of living fertilized oocytes of *Archigetes appendiculatus.* These are granular oocytes in which he described polar-body-formation as first division events. Cleavage divisions of the zygote resulted in a morula composed of all the blastomeres formed. Studies by the writer (Ogren, 1953), being prepared for publication on *Proteocephalus,* indicated that this genus with granular oocytes had cleavage stages following polar-body-formation in which all blastomeres formed the embryo. Studies on *Baerietta* by Douglas (1956) follow a similar pattern. However, the excellent studies of *Diphyllobothrium latum,* which has granular oocytes, conflict with the above as the

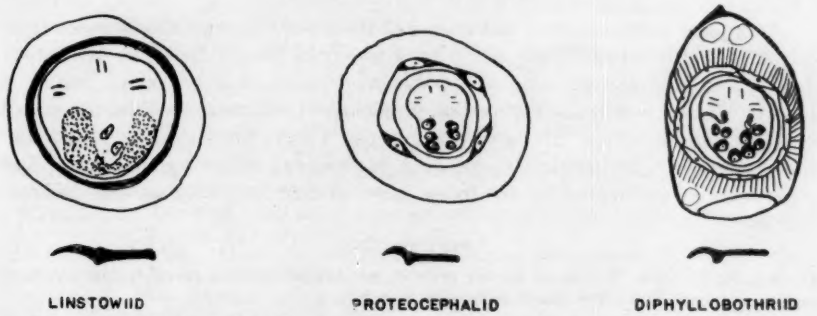
division events were interpreted by investigators (Schauinsland, 1886; Janicki, 1909; Vogel, 1929, 1930; Vergeer, 1936). In these reports, polar-body-formation was not investigated, hence, we do not know if the first cell divisions described represent meiosis or cleavage of the zygote. However, the first cell divisions they observed resulted in 2 cells, one of which moved above the vitelline cells, divided to form 2–3 small cells, and took no further part in embryogeny. The other larger cell underwent cleavage in which all blastomeres formed the embryo. The epidermis formed a ciliated embryophore (ciliaphore). If we consider the first divisions observed to be those of meiosis and the resulting small cells formed to have been polar bodies, the embryogeny of the diphyllobothriid group conforms to the generalization. Restudy of meiosis and early cleavage in *Diphyllobothrium* would settle the problem.

### *Embryo Morphology and Fate of Epidermal Cells*

The infective embryonic stage is the oncosphere. It develops in all cases from a morula of blastomeres which differentiate to form the hexacanth. Among cestodes studied by the writer the fate of the embryonic epidermis varied. At least 7 basic conditions can be recognized. Whether or not these each represent a phylogenetic series remains to be discovered. Each egg formed during embryogeny has an outer vitelline shell and an inner membrane. Oncospheres formed may differ slightly and have characteristic hooks, but in general they are similar in behavior and functions (Text-Figures 2, 3).




TEXT-FIGURE 2. The above diagrammed oncospheres with their protective membranes developed in cyclophyllidean tapeworms whose oocytes are conglomerate. Only one-half of the zygote forms the embryo. The lateral hooks shown are all drawn to the same scale from actual specimens.




TEXT-FIGURE 3. The above diagrammed oncospheres are from the tapeworms whose oocytes are granular. The entire zygote forms the embryo. The hooks are the same scale.

1. *Mesocestoidid Group*—The embryonic epidermis detaches from the inner cell mass to become a cellular membrane that eventually becomes thin and transparent. The oncosphere is quickly activated and its body is composed of muscular parenchyma cells, 6 characteristic hooks and many large plastin cells.

2. *Taeniid Group*—Development similar to *Mesocestoides*. However, the embryophore formed becomes a thick, striated membrane that obscures the embryo. The oncosphere consists of muscular parenchyma cells and a few plastin cells. The group includes *Taenia, Echinococcus.*

3. *Anoplocephalid Group*—The embryonic cells of the epidermis detach to form a pyriform or bicornuate membrane, part of which is secreted. This encloses the tiny oncosphere which consists of muscular parenchyma cells and a few large plastin cells (*Bertiella, Anoplocephala,* etc.).

4. *Hymenolepid Group*—The morula is large and the epidermal cells fail to detach from the inner cell mass. Certain epidermal cells become glandular and form the granular "penetration glands." The entire embryo becomes the oncosphere, which is usually large with large hooks and few plastin cells (*Hymenolepis, Diorchis, Choanotaenia, Rallietina*). Between the oncosphere and the shell an inner noncellular membrane, the pseudoembryophore, appears that is derived from colloidal secretions of the oncosphere.

5. *Linstowiid Group*—The embryo of this group as exemplified by *Oochoristica* is similar to the hymenolepid embryo. However, the different developmental pattern and different morphology of the epidermal glands separate it from this group. The embryonic epidermis does not separate and the entire oncosphere is the embryo.

6. *Proteocephalid Group*—The epidermal cells of the morula detach to form a cellular embryophore. This is a thin membrane, but the cells contain granular masses. There is sufficient difference in appearance to make a separate group, especially since the embryonic development results in all blastomeres forming the embryo. The oncosphere consists of muscular parenchymal cells and several very well formed plastin cells.

7. *Diphyllobothriid Group*—The characteristic embryo of this group is the coracidium. It is formed as the result of epidermal cells separating from the inner cell mass and developing cilia. The oncosphere differentiates from the inner cell mass and consists of a muscular parenchyma and several large plastin cells. Two prominent flame cells are present in this oncosphere. In the other groups, flame cells do not appear until in the metacestode stage.

### *Metacestode Morphology*

Additional confirmation of elaboration of the above 7 groups should come from comparison of metacestode stages. A brief survey of known life cycles in Cestoda does support the groups, but considerable more work of a systematic nature is needed. It may well occur that certain physiological differences will be recognized in comparing the various developmental groups. Future investigations should continue to explore the relationship between embryogeny, histology, life cycles and physiology, as exemplified by the recent paper of Smyth (1956) on egg-shell-formation.

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## DESCRIPTION OF PLATE

[All Figures made with aid of camera lucida attachment. Each scale division represents 0.01 mm at 950×. Abbreviations used: toluidine blue (TB), Lillie's metachromatic stain with acid hematoxylin and safranin 0 (MHS), methyl green-pyronin (MP)].

1. Morula stage, early oncoblasts are shown with intensely stained granules, TB.
2. Oncoblast, cross section of early hook blade in the cell, cell granules were grey, hook was pink, MHS.
3. Oncoblast, side view, beginning of blade, MHS.
4. Oncoblast, side view, older blade, MHS.
5. Oncoblast, side view, blade nearly completed, shank beginning to form, MHS.
6. Oncoblast, blade forced from the cell, shank nearly complete, MHS.
7. Dorsal-lateral hook, TB.
8. Ventral-lateral hook, TB.
9. Medial hook, TB.
10. Young oncosphere, pink epidermal glands, cortical cells appear vacuolated, MHS.
11. Young oncosphere, typical arrangement of epidermal cells, red stained contractile center or primordium, MP.
12. Young oncosphere showing young hooks, granules along thin membrane around the oncosphere, contractile center colored purple, colloidal substance in epidermal glands was green-blue, granules at surface of gland were purple, TB.
13. Muscle of oncosphere associated with hooks. The 2 black dots in center of contractile mass represent medial hooks, MHS.
14. Contractile substance associated with cells of contractile center, MHS.
15. Contractile substance associated with contractile center. This was observed best in young oncospheres, MHS.
16. Oblique section through oncosphere showing enlargement of gland area, grey colloidal material was colored green-blue, granules were purple, TB.
17. Oncosphere, C-shaped gland area, colloidal substance was pink, hooks were pink, MHS.
18. Oncosphere, older condition in which a thick pseudoembryophore has formed between shell and oncosphere. This membrane was colored red, hooks were red, large gland granules were blue-green, other cell granules were red, MP.
19. Living oncosphere in shell and inner membrane, glands were yellow, granules were active, not all cells figured, neutral red stain in Ringer's solution.
20. Oncosphere, 1 day in the beetle hemocoel, no stain, gland area with inactive granules, secreted non-cellular membrane around the oncosphere.
21. Oncosphere, mature condition, large granules in gland were blue-green. Lateral muscle bands were blue-green, other cell granules were red, MP.
22. Egg, with oncosphere enclosed by shell and pseudoembryophore, small active globules beneath shell were colored yellow and obscured the oncosphere, the pseudoembryophore was red, neutral red stain *in vivo*.
23. Living oncosphere, shell removed, yellow gland areas with active granules; colloidal material around oncosphere can be seen at hook end, neutral red stain *in vivo*.
24. Old oncosphere cut at 5 microns, stained for mitochondria after Regaud's fixation. The black masses represent cell groups with red stained cytoplasm (compare with Fig. 26). Hooks were red, pseudoembryophore was pink, cuticle was pale pink, outer shell was reticulated and contained many rod-like red stained bodies, aniline acid fuchsin procedure.
25. Old oncosphere, 5-micron section, stained with toluidine blue after Regaud's fixation. Shell was green, pseudoembryophore was purple, colloidal material between membranes was blue-green, gland area contained largly purple granules some were green blue, some purple substance was in the cuticle.
26. Old oncosphere, 5-micron section Regaud's fixation, shell was grey, pseudoembryophore was red, cuticle was grey, cell cytoplasm was brown grey, matrix was compact and colored grey, MHS.
27. Old oncosphere, AFA fixation, purple pseudoembryophore closely applied to oncosphere. Hooks and compact plastin cells were purple. Anterior part of pseudoembryophore was thinner than the posterior layer, TB.

PLATE I

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28. Section through the gland area of oncosphere, 5 microns, toluidine blue. Shell was green, pseudoembryophore was dark purple. The thin inner membranes next to the gland area were red purple, droplets at the surface of the gland were purple, large gland granules were green-blue, compact plastin cell cytoplasm was purple.

29. Oncosphere, cross section through hook region, shell omitted, pseudoembryophore was red, parenchyma was grey, plastin cells were black, hooks were red. The figure shows the position of the hooks relative to each other, and to the plastin cells, MHS.

30. Oncosphere, anterior view to show the green-blue colloidal material around the oncosphere, TB.

31. Partial view of an old oncosphere after staining with periodic acid Schiff procedure following Regaud's fixation, 5 microns. Oncosphere was opaque red, shell was blue, outer pseudoembryophore was pink while inner layer of this membrane was red.

32. Living oncosphere, 1 day in the beetle hemocoel, neutral red stain *in vivo*. Granules were not active in the C-shaped gland area. 6 plastin cells were red, the globule on the membrane was yellow.

33. Living oncosphere, 7 days in the beetle hemocoel, gland area nearly absent, increased number of plastin cells. Granules in gland were yellow. All cells could not be included, neutral red stain *in vivo*.

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## RESEARCH NOTE

### THE EFFECTS ON *SCHISTOSOMA MANSONI* OF CASTRATION AND GLUCOSE SUPPLEMENTATION IN MALE ALBINO MICE

Several investigators have reported variations in development and sex ratio for *Schistosoma mansoni* under specialized conditions. (Moore, Yolles and Meleney, 1954, J. Parasitol. **40:** 166–185; Standen, Ann. Trop. Med. Parasitol., 1953 **47:** 26–43). Others have indicated the importance of carbohydrates to the parasite, especially the male (Bueding, 1950, J. Gen. Physiol. **33:** 475–495; Bueding and Koletsky, 1950, Proc. Soc. Exper. Biol. Med. **73:** 594–596). An attempt has been made, therefore, to determine the effects of castration and of glucose supplementation on the sex ratio of *Schistosoma mansoni* in mice.

Sixty-two 5-week-old male albino mice were distributed into 4 groups, of which 32 were castrated by surgical removal of the testes at the epididymis level. The groups consisted of 22 castrated mice, 10 castrated-glucose mice, 10 uncastrated-glucose mice and 20 uncastrated control mice. The animals were infected 2 weeks after castration and 1 day following addition of 10% glucose to the drinking water. Fresh water, 10% glucose solution and Purina laboratory chow were supplied *ad libitum*. The mice were exposed percutaneously to approximately 175 pooled cercariae. Necropsies were performed 8 weeks later at which time the worms were sexed and counted. (Five mice died previous to the termination of the experiment. These consisted of two castrated, one uncastrated-control and two uncastrated-glucose animals.)

No appreciable structural changes were noted in the worms. Since the number of mice per group varied the following mean worm counts were used for comparisons.

| Mouse Group | Parasite Group Mean | |
|---|---|---|
| | Male | Female |
| 1. Castrated (20 mice) | 11.0 ± 3.16 | 12.8 ± 4.60 |
| 2. Castrated-glucose (10 mice) | 16.6 ± 4.98 | 13.0 ± 5.0 |
| 3. Uncastrated-glucose (8 mice) | 18.8 ± 3.30 | 14.5 ± 3.16 |
| 4. Uncastrated-control (19 mice) | 17.6 ± 4.09 | 13.7 ± 4.79 |

It appears from this preliminary experiment, that castration may have accounted for a considerable decrease in the mean number of male schistosomes. Further, a comparison of the mean female worm counts shows very little change for all groups. However, the castrated-glucose group shows a rather interesting recovery towards the control mean for male worms. This suggests a possible compensatory activity of the glucose supplement after castration.—EDWARD BERG, *New York State College for Teachers, Albany, New York.*

# HOST EFFECTS ON THE LIPID FRACTION OF *HYMENOLEPIS DIMINUTA**

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Previous work in this and other laboratories has indicated the presence of a surprisingly large amount of fatty material in many cestodes. However, little is known of the function, synthesis or degradation of this ether-soluble fraction in either this or other parasitic organisms. The information available at the present time consists almost entirely of gross analyses for total amounts of material in the animal and analyses for the presence of the more common lipid components (von Brand, 1952).

In order to establish a starting point for the present investigation on lipid metabolism in cestodes a preliminary postulate was made, that the lipid fraction of the worm results predominantly from the intermediary metabolism of the worm itself rather than representing a simple accumulation of materials from external sources. In the light of our knowledge of lipid metabolism in higher forms this may seem to be an obvious conclusion. However, the closeness of the host-parasite relationship permits the possibilities (1) that contributions to the lipid in the parasite may be made from the dietary intake of the host as well as (2) that the host's physiology may exert influences on the metabolic activities of the parasite. In establishing the applicability of the postulate to lipid deposition in cestodes it is apparent that determinations were required as to the importance and extent of the "host effect" on the level and nature of lipid deposition in the worm. Initially, a study on the extent of the effect of the host itself was attempted, presumably exclusive of its dietary vagaries by subjecting the parasite to the "normal" environments presented by different host types. This, it was felt, would minimize the effect of the actual contents of the lumenal environment on the lipid deposition in the worm, since all animals receive the same commercial rat food. This type of host comparison study is not without precedent as Ackert (1935), Larsh (1944, 1946), Voge (1952), Read and Voge (1954), and Read (1956) have used different strains and species of host in studying various responses by single parasitic types.

## MATERIALS AND METHODS

Adult male animals, infected with 2 cysticercoids, were used in all experiments except those involving the hamsters. The hamsters were given a dose of 5 cysticercoids; this was necessary to insure establishment of an infection approximating that found in the rats. The 5 hamsters involved in these studies averaged 2 worms each, with one animal producing 3 worms and one animal producing only 1 worm. All experimental animals were used in compiling the results except where complete infection failure occurred. The parasite larvae were raised in meal beetles (*Tenebrio molitor*) from eggs obtained from stock infections maintained in male Sprague-Dawley rats for over 6 years. Three strains of inbred albino laboratory rats (Sprague-Dawley, Sherman, and Wistar) and 1 strain of randomly bred black and white rats (Long-Evans) were employed. In order to vary the host range as much as possible the golden hamster (*Cricetus auratus*) was also tried as the host animal.

The animals were sacrificed 3 to 4 weeks after infection. At this time the host animal weight ranged from 180 to 220 g. The worms were harvested by flushing with 0.8% KCl, using

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a large (50-ml) syringe as the source of pressure. The numerous small mucoid rings encircling the parasites were removed. The worms were then blotted on filter paper and weighed on a tissue balance.

Total lipid percentages were obtained by the use of the weight loss method. In preparation for this the worms were homogenized in ground-glass homogenizers and the resulting cell-free homogenate was lyophilized. The dried material thus obtained was then placed in small tared filter beakers. These beakers and samples were dried at 80° C for 4 hours, cooled in a dessicator and weighed. The lipid extractions were accomplished by introducing 5 ml anhydrous di-ethyl ether into the beakers, followed by 10 minutes of intermittent shaking. The ether was then removed by attaching a vacuum to the side arm. This procedure was repeated 8 times in order to assure complete removal of all of the ether-soluble material. It was found that 9 or 10 extractions resulted in no further weight loss over that of 8 extractions. Following extraction the beakers and samples were dried at 80° C for 3 hours to remove any water that might have been present in the ether reagent. After cooling in a dessicator a final weight was obtained, and the percent of ether-soluble material per total dry worm weight was calculated.

Iodine numbers on the ether-soluble material were obtained by extracting pooled lyophilized material on the soxhlet apparatus for 48 hours and transferring the ether-dissolved sample to tared iodometric flasks for titration. All possible precautions were taken to assure that the extractions and iodine number determinations were handled in a comparable fashion in each of the individual experimental groups. Protection against spontaneous saturation on exposure to oxygen was maintained by keeping the samples in an ether atmosphere in tightly stoppered flasks between the time of extraction and final iodine number titration. The method used was that of Hanus, as reported by Jacobs (1951) employing a bromine-iodine-acetic acid solution as the reacting medium and standardized sodium thiosulfate as the titrating reagent. Methods of calculations were essentially those of Jacobs (1951).

## RESULTS

In the initial stages of the study it was found that the parasite could be main-

**TABLE I — *Fresh weight of worms from various host animals***

**The mean and the standard deviation for each host group in labeled squares. Difference between means and the standard error of these differences in intersecting squares. (Figures in parenthesis indicate number of animals and number of worms used in determinations):**

| | | | | |
|---|---|---|---|---|
| | | | | Hamster (5;10) 529 ± 33 |
| | | | Long-Evans (7;14) 709 ± 38 | 180 ± 57.4* |
| | | Sherman (5;10) 847 ± 32 | 138 ± 49.6† | 318 ± 53.6* |
| | Wistar (6;12) 991 ± 64 | 144 ± 71.5† | 282 ± 74.4* | 462 ± 77.1* |
| Sprague-Dawley (14;28) 636 ± 29 | 355 ± 63.9* | 211 ± 33.1* | 73 ± 47.8† | 107 ± 41.9† |

*Difference significant.
†Difference not significant

tained with no difficulty in the several rat hosts used and that the resulting mature worms were quite similar in gross appearance and number in the individual hosts. However, in the hamster infections, the number of worms resulting from a known cysticercoid dose was less and the general appearance of the worms, etc. was much less consistent. Within the methods used in this laboratory the guinea pig proved to be refractory to infection with this parasite.

*Fresh Worm Weights:* Table I gives the mean fresh weight of worms from these host animals. In intersecting squares are given the differences between means of the various host animal groups. It will be noted that the difference found between the worms from the Sprague-Dawley and the Long-Evans groups was not significant. This was also true for the differences between the Sprague-Dawley rats and the hamsters, the Sherman and the Wistar rats, and the Sherman and Long-Evans rats. However, the differences shown between the other host animal groups were found to be significant. The hamsters generally produced the smallest worms while the Wistar rats produced the largest.

*Total Percent Ether-soluble Material Dry/worm Weight:* Data on the total amounts of ether-soluble material in the same worms used in the above study are

**TABLE II — *Ether soluble percentage of dry worm weight from various host animals***

| The mean and the standard deviation shown in the labeled squares. Differences between means and the standard error of these differences shown in intersecting squares. (Figures in parenthesis indicate number of animals and number of worms used in determinations). | | | | |
|---|---|---|---|---|
| | | | | Hamster (5;10) 9.52 ± .50 |
| | | | Long-Evans (7;14) 16.45 ± .37 | 6.93 ± .62* |
| | | Sherman (5;10) 12.43 ± .54 | 4.02 ± .67* | 2.91 ± .73* |
| | Wistar (6;12) 12.60 ± .33 | 0.17 ± .63† | 3.85 ± .49* | 3.08 ± .59* |
| Sprague-Dawley (14;28) 15.21 ± .42 | 2.61 ± .53* | 2.78 ± .69* | 1.24 ± .56† | 5.67 ± .65* |

* Difference significant.
† Difference not significant.

presented in Table II. It may be seen that a more consistent range of percentage fat was found in worms produced by the different groups of host animals than was found for fresh worm weights. It will also be noted that the significance of the differences between the means of all the host animal groups was more extensive, excepting only the Sprague-Dawley vs. Long-Evans rats and the Sherman vs. Wistar rats. These same 2 latter groups also failed to demonstrate a significant difference when the fresh worm weights were compared (Table I).

In a comparison of the data from Table I and Table II, 2 relationships are evident. First, the worms from the Sprague-Dawley and the Long-Evans rats, which, of the rat groups, gave the lowest average fresh weights, showed on the other hand the highest average content of ether-extractable material. Second, the worms from the Wistar and Sherman rats, which showed the highest average wet weights, produced in contrast, the lowest amount of ether-extractable material. Only the worms from hamsters were consistently low in both categories.

*Saturation of Incorporated Fatty Acids:* Since it was apparent that the percentage of fat material in the parasite varied to a measurable and statistically significant extent when certain host animals were used, efforts were made to determine whether the worms from the different host groups differed in their lipid content qualitatively, as well as quantitatively. Groups of worms from similar host animals were pooled and extracted on the soxhlet extraction apparatus. With this method a sufficient amount of the fat fraction of the worm could be obtained for Hanus iodine number determinations. The figures so obtained may not be considered values indicative of the actual number of double bonds present in the individually involved fatty acids. However, they do give a criterion for a comparison of the worms in terms of the relative saturation of their total ether-extractable fraction. The data given in Table III represent values obtained as the result of 2 completely separate analyses made 6 months apart.

TABLE III — *Hanus iodine number of fat fraction of worms from various host animals*

| Host Animal | Hanus Iodine Value Determ. I | Determ. II |
|---|---|---|
| Sprague-Dawley Rats | 140 | 151 |
| Wistar Rats | 110 | 94 |
| Sherman Rats | 200 | 218 |
| Long-Evans Rats | 90 | 87 |
| Hamsters | 140 | 147 |

The information presented in Table III indicates that the pattern of intermediary metabolism in the worm responsible for the degree of saturation of the fatty acids incorporated into the lipid fraction of the worms was, in some way, dependent on the environments presented by the different species and strains of host animals used. The complexity of the host effect on the metabolism of *H. diminuta* is apparent from the variability in these effects on different aspects of tapeworm metabolism as was seen in the comparison of Table I and Table II. It is further emphasized by a comparison of Tables II and III. It will be noted that in terms of total ether-extractable material the Sprague-Dawley group of worms and the Long-Evans worms were quite similar. This similarity did not apply when a

comparison was made of the final combinations of saturated and unsaturated fatty acids contributing to the total lipid component of the worms. This same inconsistency was found to be true of the Sherman and Wistar groups of worms.

## DISCUSSION

The influence of various hosts on the size attained by the rat tapeworm, *H. diminuta,* as reported by Read and Voge (1954) is confirmed in the case of the 3 host animals used in common in their investigation and in the present one. Using volume displacement, Read and Voge reported figures of 0.57 ml for worms from hamsters; 1.11 ml for worms from Sprague-Dawley rats and 1.33 for worms from Long-Evans rats. In the present report fresh weight determinations for worms from similar hosts gave values of 529 mg, 636 mg, and 709 mg respectively.

The deposited lipid material found in *H. diminuta* is apparently a rather sensitive indicator of environmental variations. As was seen above, not only can the total amount of this fraction be varied significantly by changing the species or even strain of host animal used, but also present indications are that the nature of the fat may be altered. As in the case of many other experimental procedures using cestodes, a marked individual variability was encountered. In the present situation this variability probably came from an inability to control the shedding of proglottids by the worm, combined with individual worm and host physiological variations.

It is also difficult to interpret these results in terms of the biology of the tapeworm, at present there is too little information concerning either the mechanisms that are used to produce this lipid material or the role that the latter may have in the nutritional economy of the worm. Indeed, information concerning the intermediary lipid metabolism of fats in invertebrates, as a whole, is extremely meager. In the light of our knowledge of fat synthesis and the interconversion systems in mammalian tissues, however, certain facts that have been disclosed from time to time concerning cestode metabolism suggest possibilities. Previous work has indicated a very high rate of glycolysis for this parasite (Read, 1950). In addition, other workers, as well as the present authors, have found that an *in vitro* cultivation of *H. diminuta* for any length of time is difficult due to the relatively large amounts of organic acids that are rapidly produced even under aerobic conditions. There is still some question as to the anaerobic or aerobic nature of the general environment for this cestode but the rate of production of organic acids by the tapeworm regardless of environment infers that the activity of the tricarboxylic acid cycle is limited, as an effective metabolic pathway. The accumulation of organic acids, including acetic acid, makes it likely that the lipid material found in this parasite may result from a mechanism employing these organic acids as starting materials. Several studies are under way, the results of which should shed additional light on the extent to which this actually occurs. In view of these possibilities it is not completely unlikely that the effect of the different hosts on fat deposition in the tapeworm may have resulted in part from influences brought to bear on the glycolytic-glycogenesis system of the worm. In this regard it may be pointed out that Read (1956) and Daugherty (1956) arrived at quite dissimilar results in studies on glycogenesis in *H. diminuta* but used quite different experimental conditions, including a different rat strain.

The role of the fat, after its synthesis, is quite uncertain. Certain features

of the previous discussion, however, suggest that the actual synthesis of the fat may serve the tapeworm as a means of "buffering" the rapidly formed organic acids by chemical synthesis to neutral fats. This would, indeed, be of great service to the worm. There is no information at present that suggests that the fat itself is usable to the worm for either glyco-neogenesis or as a source of energy.

One of the results of this work, where quantitative measurements are concerned, is the reaffirmation of the imperative need for an absolute identification and control not only of the experimental factors pertaining to the parasite, but also of those pertaining to the host animal in which it is cultivated. This is particularly true when the investigation is concerned with intermediary metabolism, as these chemical reactions are the factors that determine the larger manifestations which appear as the result of changes in the host-parasite relationship.

SUMMARY

The rat tapeworm, *Hymenolepis diminuta*, when grown in different species and strains of host animals, showed differences in the fresh weight of worms produced. Variations were also found in the total amount of ether-soluble material present in these worms. The degree of saturation of the fatty acids or the combination of saturated and unsaturated fatty acids incorporated into the lipid fraction of the worms was found to respond to the environmental variations in the gut of the different hosts. The significance of these results and the importance of host animal definition in parasite physiology studies are discussed.

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# GLYCOGEN DISTRIBUTION IN CYSTICERCOIDS OF THREE HYMENOLEPIDID CESTODES

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Adult tapeworms are known to contain relatively large amounts of glycogen. Glycogen content of *Hymenolepis diminuta* has been investigated by Read (1949, 1956) and by Daugherty and Taylor (1956). Recently, the presence of glycogen was reported for the scolices of larval *Echinococcus granulosus* (Agosin *et al*, 1957). It was therefore of interest to determine the presence or absence of polysaccharide in the larval stages of *H. diminuta* and related species. Furthermore, it was postulated that the tail of the cysticercoid larva might function in the storage of glycogen.

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## MATERIALS AND METHODS

The species of tapeworms employed in this study were *Hymenolepis nana, H. diminuta,* and *H. citelli.* The Rice Institute strain of *Hymenolepis diminuta* was maintained in laboratory rats, and the *H. nana* strain from the Rice Institute in mice. In addition, a strain of *H. nana* was obtained from infected Webster albino and $DBA_1$ Swiss mice purchased locally. *H. citelli* was obtained locally and maintained in ground squirrels. *Tribolium confusum* raised in enriched flour at 30° C served as intermediate hosts. Egg suspensions were placed on filter paper and fed to starved adult beetles for 4–6 hours. The beetles were then transferred to flour in fresh containers and placed in incubators kept at temperatures ranging from 20°–37° C.

After removal of the cysticercoids from the body cavity of their hosts, the living larvae were stained with Gram's or Lugol's iodine. Cysticercoids were also fixed in a variety of fixatives and stained as whole mounts with a permanent iodine stain, Best's carmine, or Periodic Acid Schiff stain. Paraffin sections were cut at 10 microns from material fixed in a solution consisting of 9 parts absolute ethyl alcohol and 1 part formalin and stained with Best's carmine. Some of the sections were digested in 1% malt diastase for 1 hour at 37° C, and stained subsequently as directed by Lillie (1953).

## RESULTS

When iodine is added to fully developed, living cysticercoids, grown at 30° C, some portions of the body stain a deep reddish-brown. Figures *a* and *c* (Plate I) illustrate living, unstained cysticercoids of *H. nana* and *H. diminuta.* It should be noted that the scolex proper lies below the pair of dark, central masses which are concentrations of granular material. The membrane which encloses the scolex also surrounds this material. Figures *b* and *d* show the same specimens after the addition of a drop of Lugol's iodine. An intense reaction to the stain is observed in the tail and scolex of both species, and at the body-tail junction of *H. diminuta.* The outermost cysticercoid membranes and associated parenchymatous tissues do not stain. In *H. citelli,* the staining reaction is very similar to that illustrated for *H. diminuta.* While the staining reaction in the tail is almost instantaneous, it is considerably slower in the scolex, which is surrounded by several enveloping layers which must be penetrated by the staining solution.

Microscopic examination of cysticercoids of *H. diminuta* and *H. nana,* stained with iodine, showed that the vacuolated areas of the tail as well as the peripheral layer of cells always remained unstained. Tail vacuoles (swollen portions of tail

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of *H. nana,* Plate I, a, b) are not present in the specimen of *H. diminuta* illustrated in Figures c and d. The scolex region of the two species appears uniformly dark in the whole-mount stains both in the scolex proper and in the inner cellular layer which immediately envelops the scolex and is continuous with it at the base. Permanent preparations of whole mounts stained with iodine, Best's carmine, or PAS always showed the same general distribution of the stain. Undigested sections stained with carmine reacted as did the fresh material in iodine. Localization of

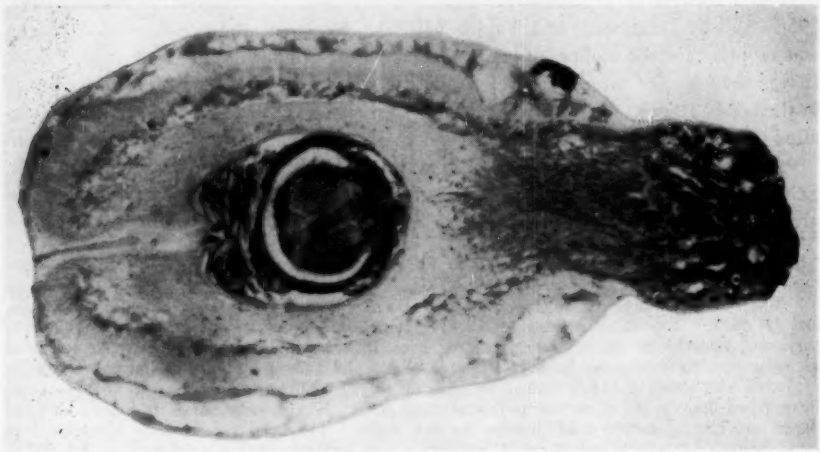

FIGURE 1. Section of cysticercoid of *Hymenolepis diminuta* stained in Best's carmine showing localization of glycogen. Highest concentration is seen in the tail (only partially shown), the scolex, and the tissues immediately surrounding the scolex and continuous with it.

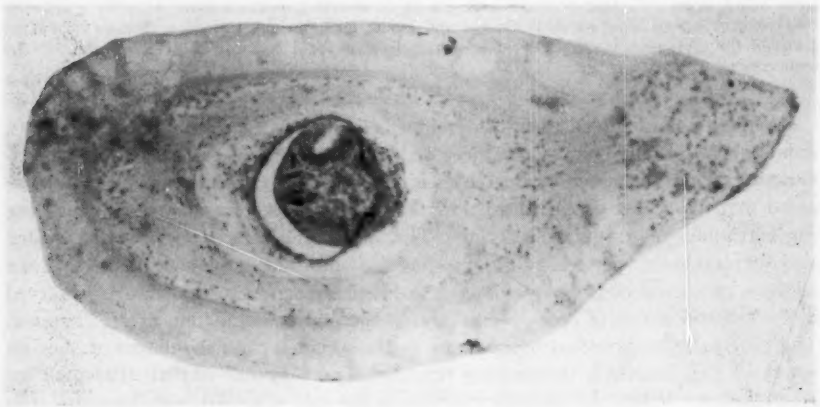

FIGURE 2. Section as in Figure 1, but digested in 1% malt diastase at 37° C before staining with Best's carmine. Note absence of heavy stain shown in Figure 1.

the stain is clearly seen in the parenchyma of the tail (only partially shown in sections) in the muscle cells of the suckers, in the basal portion of the scolex (future neck region), and in the layer continuous with the neck which forms the inner enveloping sheath about the scolex (Fig. 1). Sectioned material stained with Best's carmine after digestion with malt diastase did not stain except for an overall pinkish cast (Fig. 2). It is therefore reasonable to assume that the tail and parts of the scolex and surrounding tissues contain polysaccharide which is probably glycogen.

After the results obtained with fully developed cysticercoids, developmental stages of both *H. nana* and *H. diminuta* were examined for the presence of glycogen. It was found that the amount of this material increased with growth and development of the larvae. Small amounts appear very early, before elongation of the larvae begins. Just prior to withdrawal of the scolex, greater concentrations of glycogen are observed in the tail, in the parenchymatous cells immediately surrounding the large central cavity, and in the everted scolex, particularly the zones of sucker differentiation, the anterior margin, and the neck region in contact with the main body of the cysticercoid.

Since different environmental temperatures have been shown to influence markedly the speed of cysticercoid development (Voge and Turner, 1956; Heyneman, in press) it was thought that the amount of glycogen stored might differ in larvae which had undergone slow or very rapid development. Cysticercoids of both *H. nana* and *H. diminuta* were therefore grown at 20, 30, and 37° C and the results compared. No marked differences in glycogen distribution were observed between larvae grown at 37° or at 30° C. However, since tail-size is much smaller in larvae which have developed at 37°, the total amount of glycogen is considerably less. Glycogen distribution in cysticercoids of *H. nana* grown at 20° C was very similar to that in corresponding stages kept at 30° C. However, in *H. diminuta* kept at 20° C, the tail portion contained relatively very little glycogen although the tails were long and well developed. It was then attempted to determine whether age of fully developed cysticercoids kept at any given temperature would influence the distribution or amounts of glycogen present. No differences could be observed in *H. diminuta* kept at 30° C for 8 days or for 30 days. Similarly, *H. nana* cysticercoids kept at 30° C showed as intense a staining reaction after 94 days as they did after only 20 or 30 days. When kept at 37° C, the staining reaction was as intense after 18 days as it was in newly developed larvae after 4–5 days. In *H. nana*, the staining reaction in the tail of the oldest specimens, which commonly show greatly distended tails, often showed a glycogen distribution corresponding to the location and shape of the *original* tail in the newly matured, 5-day-old specimen.

## DISCUSSION

From observations reported here it appears that the cysticercoid tail in the species investigated contains large amounts of glycogen. Whether or not this material is used and replenished in the fully grown larva within the intermediate host is not known. Glycogen in the scolex may well be utilized during and immedately after attachment in the small intestine of the definitive host. Since larvae with or without tail are apparently equally infective, glycogen in the tail may not be essential for this purpose.

It should be noted that tail growth apparently continues throughout the life of the parasite in the intermediate host. However, in older *H. nana* cysticercoids kept at 30° C, there was some evidence that this growth was not accompanied by deposition of glycogen in the zone of new tail growth.

The relative importance of the cysticercoid tail in development is still uncertain. When one considers the shrinkage of the tail in *Cittotaenia* (Stunkard, 1941) and in *Monoecocestus sigmodontis* (Melvin, 1952) in contrast to the continued growth of the tail in *M. americanus* and *M. variabilis* (Freeman, 1952), it is clear that the role of this organ is far from being understood.

SUMMARY

The presence of polysaccharide, in all probability glycogen, has been demonstrated by histochemical methods in cysticercoids of the tapeworms *Hymenolepis nana, H. diminuta,* and *H. citelli.* A high concentration of this material is seen in the tail parenchyma, in the scolex musculature and neck of the larva as well as in the inner cellular layer around the scolex and continuous with it. Little or none is seen in the outer layers which form the bulk of the protective tissues surrounding the scolex. Glycogen is also absent from the central or posterior mass of vacuolated cells in the cysticercoid tail.

Observations on different developmental stages of the larvae indicate that the amount of glycogen increases as development and growth proceed. Larvae grown at different temperatures ranging from 20°–37° C did not show any significant differences in the distribution of glycogen, with the exception of *H. diminuta* in which, when grown at 20° C, the amount of glycogen in the tail of the cysticercoids was very much reduced.

Comparison of newly-matured larvae with larvae as old as 90 days did not show any differences in amount or distribution of glycogen.

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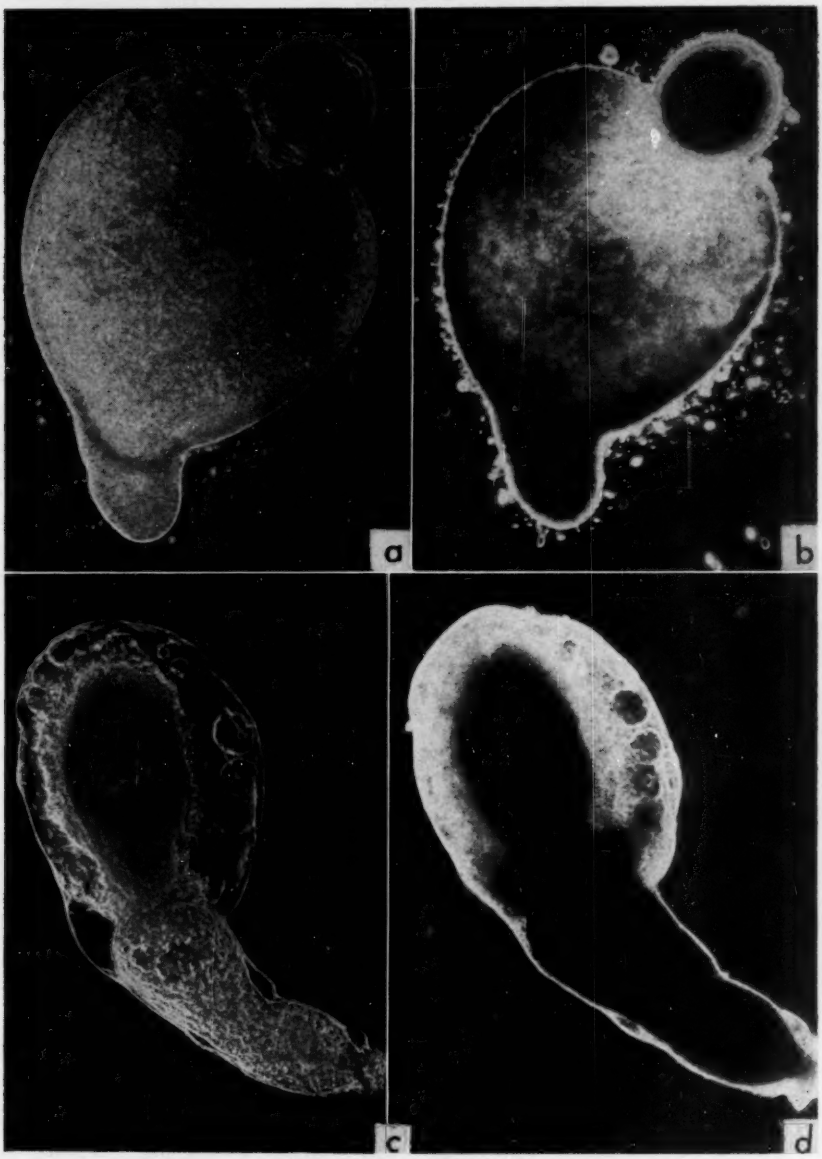
PLATE I




EXPLANATION OF PLATE I

(All photographs darkfield, approximately ×83)

FIGURE a. Living cysticercoid of *Hymenolepis nana*, shortly after removal from *Tribolium confusum*.

FIGURE b. The same specimen as in figure a, shortly after the addition of Lugol's iodine. Dark areas are zones of heavy glycogen concentration.

FIGURE c. Living cysticercoid of *H. diminuta*, after removal from *Tribolium confusum*.

FIGURE d. The same specimen as in figure c, shortly after the addition of Lugol's iodine. Dark staining areas are zones of heavy glycogen concentration.

# FREEZING PROCEDURES FOR GREATER FLEXIBILITY IN APPLICATION OF THE DIGESTIVE METHOD FOR POST-MORTEM RECOVERY OF CATTLE NEMATODES.

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It is generally accepted that not all the nematodes present in the alimentary tract of cattle are recovered through conventional methods of post-mortem examination, even when the inner wall is thoroughly scrubbed and rinsed. Some of the nematodes, especially the immature worms, are either embedded in or firmly attached to the mucosa.

In an attempt to obtain a more accurate estimation of the nematode population of the alimentary tract of experimental cattle at the Georgia Experiment Station, it was decided to utilize the digestion technique advocated by Herlich (1956) for the recovery of embedded and adherent worms. The addition of the number of worms obtained by this method to the number obtained by preliminary rinsing would offer a practically complete determination of the worm load. Material so treated must be examined shortly after completion of the digestive process in order to prevent any detrimental effect on the worms by the medium. Since the number of cattle necropsied at one time is usually too large to permit such prompt examination, an experiment was designed to determine the practibility of (1) freezing the material obtained by digestion of the fresh organs and maintaining it in that state for later examination, and (2) freezing the fresh digestive tract and keeping it in that state for some time prior to its digestion and subsequent freezing.

## MATERIAL AND METHODS

Three rabbits were each infected with approximately 15,200 third-stage larvae of the small stomach worm of cattle, *Trichostrongylus axei*. The host animals were killed 5 days later because it was thought that if the diagnostic morphology of the larval, or immature stages then present was found not to be materially damaged by freezing, adults undoubtedly would be identifiable after congelation. The stomachs were removed immediately and rinsed individually to remove the ingesta, which was examined for larvae.

The stomach from rabbit 3993 was weighed and placed in a beaker, to which a proportionate amount of digestive fluid was added (600 ml of water, 10 ml of concentrated HCl, and 5 gm of pepsin for each half pound of material to be digested). Digestion was carried out in an incubator at 37°C until complete (about 4 hours). The stomachs from rabbits 3994 and 3995 were placed in separate containers and inserted in the freezing unit of a refrigerator as 0°C, where they remained for 3 and 7 days, respectively, before being thawed and digested.

The material obtained by digestion of each of the 3 stomachs was sampled as follows: The total fluid volume of the contents of the beaker was determined. By agitation, the larvae present were uniformly suspended in the fluid, and 12 aliquots of the suspension were taken and placed in glass vials. Each aliquot represented a known fraction of fluid. Thus, the total number of larvae present in any of the stomachs was calculated from each of the aliquots as the product of the number of larvae recovered and the dilution factor represented by the aliquot. Two of the aliquots served as controls. They were examined immediately; the worms in each were counted and scrutinized for evidence of degenerative changes. The remaining 10 aliquots were frozen; five were first diluted with water to see if freezing in a lowered concentration of the digestive fluid would be less deleterious to the larvae. The test aliquots were removed from the freezing unit at irregular intervals and thawed, and the worms in them recovered and counted under a dissecting microscope and studied with the aid of a binocular research micro-

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scope after fixation. A solution of 10% iodine and 95% alcohol was added by means of a dropper to the material until it turned to a dark-brown color. The larvae were more heavily stained than the surrounding medium, thus facilitating their detection against a light background. This staining technique also proved satisfactory for adult worms, and further work along this line is being pursued.

## RESULTS

Examination showed that the viability of the larvae in the control aliquots of the material obtained by digestion of the stomachs was as follows: When the stomach was digested immediately after necropsy (rabbit 3993), all were alive; when the stomach was frozen for the 3 days prior to digestion (rabbit 3994), 6% were alive; when the stomach had been frozen for 7 days prior to digestion (rabbit 3995), none were alive.

There was no evidence of any morphological change in the larvae from the frozen aliquots, or any other condition which indicated a possible deteriorating effect of the digestive medium, diluted or undiluted, during the maximum period (112 days) of freezing tested (Table I). A slight increase in the transparency

TABLE I— *Total numbers of* Trichostrongylus axei *larvae estimated from each of the control* and frozen† aliquots of the digested stomachs of three rabbits*

| Days Aliquot Kept In Freezer | Digested On Day Of Necropsy (No. 3993) | | | Digested After Freezing For 3 Days (No. 3994) | | | Digested After Freezing For 7 Days (No. 3995) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control Aliquots | Frozen Aliquots | | Control Aliquots | Frozen Aliquots | | Control Aliquots | Frozen Aliquots | |
| | | Undiluted | Diluted | | Undiluted | Diluted | | Undiluted | Diluted |
| | 1,162 | | | 2,260 | | | 1,120 | | |
| 0 | 1,778 | | | 2,380 | | | 1,080 | | |
| 1 | | 1,064 | 1,330 | | 1,640 | 2,400 | | 760 | 760 |
| 4 | | 1,414 | 1,694 | | 2,260 | 1,920 | | | |
| 7 | | | | | | | | 1,120 | |
| 8 | | | | | | 2,320 | | | 1,420 |
| 9 | | 1,316 | 1,274 | | 2,520 | | | 1,000 | |
| 10 | | | | | | | | | 960 |
| 14 | | | | | | | | 1,300 | |
| 16 | | 1,862 | 1,960 | | | | | | |
| 24 | | | | | 2,170 | | | | |
| 30 | | | | | | | | | 1,060 |
| 31 | | | | | | 2,620 | | | |
| 33 | | | | | | | | 1,180 | |
| 44 | | | 1,540 | | | | | | |
| 105 | | | | | | | | | 1,160 |
| 110 | | | | | 2,360 | 2,440 | | | |
| 112 | | 1,442 | | | | | | | |
| Average of total worm load | 1,470 | 1,420 | 1,560 | 2,320 | 2,190 | 2,340 | 1,100 | 1,072 | 1,072 |

*Examined immediately after completion of the digestion

†Examined at the indicated intervals following digestion and freezing

of the larvae, which was also evident in larvae examined immediately after digestion was completed, was noted, but this condition did not cause any difficulty in their identification. The variation observed in the number of larvae recovered from the aliquots (Table I) was, therefore, apparently not due to the effect of the digestive medium upon the worms but to sampling error, which was probably inherent in the method used. This was indicated by the fact that oftentimes more larvae were recovered from samples frozen for a long period of time than from those frozen for a relatively short period. Moreover, variation in the number of larvae was also apparent in the control samples examined immediately after digestion.

Variation in the number of worms recovered from the different stomachs probably indicated that unequal levels of infection were established, even though the animals used were of the same age and received the same dose of larvae. Nevertheless, it can be concluded, on *prima facie* evidence, that good results may be obtained either from frozen samples of the material obtained by digestion of the organ in the fresh state, even if they are kept frozen for many weeks, or from digestion of an organ that has been preserved by freezing for a week or less.

After this experiment was completed, the stomach from a rabbit harboring *T. axei* was digested to determine whether the digestion-freezing technique would have any adverse effect on adults of this species. No significant effects were noticed on the number or morphology of the worms recovered from control aliquots or from those frozen for 3 weeks. In addition, the fresh stomachs and small intestines from 42 yearling calves were digested, and 100-ml aliquots from the digested organs were frozen in a home freezer and stored at −5°C. Examination of these samples has failed to show any morphological changes in the worms recovered, even in samples that had remained frozen for 88 days.

## SUMMARY

An experiment was conducted to determine if a recently described digestion method for the post-mortem recovery of nematodes from the wall of the digestive tract of ruminants would give accurate results when applied to (1) an organ that had been quick-frozen for a week or less, or (2) samples of material promptly frozen after digestion of the fresh organ which were kept in a freezer until they could be examined at a more convenient time. The test materials were stomachs from rabbits infected with *Trichostrongylus axei* larvae. The results showed that the number of worms recovered was not affected by the application of either or both of these freezing procedures. Digested material was maintained in a frozen state for as long as 112 days and no deleterious effect on the morphology of the parasitic larvae was detected, nor was there any apparent reduction in the number of larvae recovered. This digestion-freezing technique has been used to determine the kinds and numbers of worms in the stomachs and small intestines of 42 calves with satisfactory results.

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# THE MALE, NYMPH, AND LARVA OF *IXODES BRUNNEUS* KOCH (ACARINA: IXODIDAE)

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*Ixodes brunneus* Koch was described from North America by C. L. Koch in 1844 on the basis of the female only and without designation of host. In 1847, he listed the host as *"Fringilla albicollis"* = *Zonotrichia albicollis*. For nearly 100 years, only females were found, of which there are now many specimens in tick collections. However, it was not until 1943 that one of the authors (C.N.S.) with the assistance of Harry K. Gouck succeeded in rearing this species in the laboratory. This made all stages of the life cycle available for description. The female is here redescribed and figured for the sake of completeness.

*Ixodes brunneus* has been taken only from birds and its occurrence on many migratory birds would explain its wide dissemination. With the exception of 1 collection from Venezuela (Cooley and Kohls, 1945: 211), it has not been collected outside of the United States. In this country, it has been recorded from Massachusetts, Connecticut, New York, New Jersey, Maryland, Alabama, Arkansas, California, Georgia, North Carolina, Oklahoma, South Carolina, Tennessee, Virginia, Oregon, Texas, Mississippi and Pennsylvania.

According to Bequaert (1946: 143), with whom the authors agree, the species referred to and figured as typical *I. brunneus* occurring on Old World birds (Nuttall and Warburton, 1911: 189) is obviously some other species; there is also some doubt as to whether *I. kelloggi* Nuttall and Warburton, 1908, is actually a synonym of *I. brunneus*.

The following descriptions are based on specimens from the colony reared at the Entomology Research Branch, Agricultural Research Service, Orlando, Florida. All measurements are in millimeters.

## *Ixodes brunneus* Koch, 1844
### Male (Plate I, A–J)

*Body.* Oval, widest just anterior to spiracular plate. Seven specimens, exclusive of capitulum, from 1.90 to 1.67 long, average 1.76 and from 1.67 to 1.20 wide, average 1.26.

*Scutum.* Brown, with well sclerotized areas darker brown; surface decidedly convex with posterior third more declivous than anterior two-thirds; varies from smooth, with distinct punctations, to granulated, crazed surface, with punctations tending toward confluency. Pseudoscutum roughly outlined by anterior and lateral margins of deep depressions on each side of scutum; these 2 depressions appear deeper and more pronounced in some specimens. Scapulae short and rounded. Cervical grooves as valleys separating median and lateral areas. Marginal grooves deep and continuous posteriorly. Setae few and fine; limited to posterior region.

*Ventral plates.* All, except anal plate, well sclerotized and separated from each other by soft portions of integument; very small, numerous, sclerotized circular plaques interspersed in soft portions of integument. Pregenital plate distinct but variable in size and shape; anterior and posterior margins concave; lateral margins convex; confluent punctations. Median plate longer than wide; widest at level of spiracles; posterior margin more or less rounded; medium punctations, with few inconspicuous setae. Epimeral plates variable in size and shape; contiguous to posterior margin of spiracles; widest anteriorly tapering to a point posteriorly; lateral margins irregular; may be present as several, small irregular plates. Adanal plates variable in size and shape; widest anteriorly, tapering to a point posteriorly; internal margins concave,

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external margins convex. Anal plate weakly sclerotized; in some cases reduced to irregular ring opened posteriorly or outlined by groove enclosing numerous, small sclerotized plaques.

*Spiracular plate.* With goblets medium and numerous; for 7 specimens from 0.26 to 0.19 in dorso-ventral axis, average 0.24 and from 0.33 to 0.28 in antero-posterior axis, average 0.30.

*Genital aperture.* Opposite level of coxae II and III.

*Legs.* Very long and slender; tarsus I for 5 specimens from 0.88 to 0.50, average 0.73; tarsus IV from 0.73 to 0.57, average 0.64. Coxa I with 2 equal spurs; coxae II and III with external spur larger than internal spur; coxa IV with external spur only.

*Capitulum.* Seven specimens, from tip of palpi to tip of cornuae, from 0.43 to 0.37, average 0.40; width of basis from 0.28 to 0.25, average 0.26. Basis widest at base of palpi and narrows posteriorly. Posterior margin straight. Cornuae absent. Palpi short and wide with article 2 slightly wider than article 3. Dorso-ventral ridge on article one absent.

*Hypostome.* Length for 6 specimens from 0.22 to 0.17, average 0.19; apical portion deeply notched; dentition 3/3 with approximately 6 to 8 teeth per file; somewhat crenulate in appearance.

### Female (Plate II, A–J)

*Body.* Ovoid, widest just anteriorly to spiracles and narrowest at anterior end. Six unengorged specimens, exclusive of capitulum, from 2.22 to 1.93 long, average 2.12 and from 1.42 to 1.15 wide, average 1.31. Five specimens, fully to partially engorged, exclusive of capitulum, from 9.37 to 3.38 long, average 6.73 and from 7.00 to 2.28 wide, average 4.94. Marginal grooves well defined laterally, but not complete in posterior region of body. Postscutal area with numerous, long, thick, white setae. Two large depressed areas just posterior to scutum. Ventrally with setae and punctations as on dorsal surface. Anal groove horse-shoe shaped.

*Scutum.* Eleven specimens from 1.45 to 1.23 long, average 1.37 and from 1.07 to 0.83 wide, average 0.94; brown with well sclerotized areas and posterior margin darker brown. Median-posterior portion of scutum convex; surface irregular and shiny; punctations small to medium with tendency to confluency but absent in depressed areas. Setae similar to those of postscutal area; located on postero-median area and lateral margins but absent in depressed areas. Cervical grooves as depressed areas extending nearly to the postero-lateral margins, with area between and lateral to them raised.

*Spiracular plate.* Slightly elevated with goblets small and numerous; for 11 specimens from 0.38 to 0 34 in dorso-ventral axis, average 0.36 and from 0.35 to 0.30 in antero-posterior axis, average 0.32.

*Genital aperture.* Opposite level of coxae III and IV.

*Legs.* Long and large. Tarsi long and tapering; tarsus I for 9 specimens from 0.78 to 0.70 long, average 0.73; tarsus IV from 0.66 to 0.52 long, average 0.63. Coxa I with 2 spurs, internal slightly narrower and directed mesially; coxae II and III with pronounced external spur and with suggestion of internal spur; coxa IV with external spur only. Trochanters I to III with distinct ventral spurs and trochanter IV with reduced ventral spur.

*Capitulum.* Ten specimens, from tip of palpi to tip of cornuae, from 0.88 to 0.75, average 0.83; width of basis for 11 specimens from 0.50 to 0.40, average 0.48. Basis with posterior margin slightly concave; surface crazed. Cornua short and bluntly rounded. Porose areas large, superficial, with indistinct margins and the area between them depressed. Palpi long and narrow with external margin nearly straight and internal margin convex; palpal article 2 much longer than article 3; palpal article 1 with a dorsal and ventral ridge. Ventral surface of basis constricted below level of auriculae. Auriculae large lateral extensions, slightly variable in shape, even on same specimen.

*Hypostome.* Length for 7 specimens from 0.48 to 0.42, average 0.45; narrow and pointed; dentition 4/4 becoming 3/3 and 2/2 near the base.

### Nymph (Plate III, A–H)

*Body.* Ovoid, widest anteriorly to the spiracles. Six unengorged specimens, exclusive of capitulum, from 1.15 to 1.08 long, average 1.11 and from 0.77 to 0.70 wide, average 0.74. Four specimens, fully to partially engorged, exclusive of capitulum, from 2.25 to 1.63 long, average 1.83 and from 1.58 to 1.12 wide, average 1.30. Marginal groove distinct. Numerous thick white setae on postscutal region. Ventrally with fewer setae. Anal groove horse-shoe shaped.

*Scutum.* Ten specimens from 0.58 to 0.52 long, average 0.54 and from 0.55 to 0.47 wide, average 0.51; widest at level of coxae II and narrowed posteriorly. Surface smooth with few, small, shallow punctations and with few inconspicuous setae. Scapulae short and rounded. Cervical grooves shallow, as valleys separating median and lateral areas, terminating near postero-lateral margin.

*Spiracular plate.* For 10 specimens from 0.18 to 0.17 in dorso-ventral axis, average 0.17 and from 0.19 to 0.16 in anteroposterior axis, average 0.17.

*Legs.* Tarsus I for 10 specimens from 0.41 to 0.35, average 0.38; tarsus IV for 9 specimens from 0.32 to 0.26, average 0.29. Coxa I with 2 spurs, internal slightly longer and narrower than external; coxae II–IV with external spur only; longer than corresponding spur on coxa I.

*Capitulum.* Ten specimens, from tip of palpi to tip of cornuae, from 0.32 to 0.26, average 0.28; width of basis from 0.22 to 0.20, average 0.21. Basis with posterior margin convex and surface lightly crazed. Cornuae large and directed laterally. Palpal article 3 slightly longer than article 2; lateral margin of article 2 indented and narrowed about 1/3 distance from base; line of separation between article 2 and 3 faint; setae long and distinct. Basis long ventrally; constricted below mid-length; posterior margin rounded. Auriculae as large, lateral projections; margin of basis above auriculae projecting laterally, terminating in a point and giving impression of two auriculae on each side.

*Hypostome.* Length for 5 specimens from 0.17 to 0.15, average 0.15; narrow and pointed; dentition first 3/3 then 2/2 at base; lateral denticles large and pointed.

## Larva (Plate IV, A–B)

*Body.* Subcircular, widest slightly below midlength and narrowest at anterior end. Five unengorged specimens from a single lot, exclusive of capitulum, from 0.53 to 0.50 long, average 0.52 and from 0.46 to 0.42 wide, average 0.44. Sensilla sagittiformia absent. Twenty-six setae on dorsal surface of body, exclusive of scutum: 14 distributed around the lateral margin; 10 arranged in 2 files of 5 in the central region but occasionally with 12 or with 8 instead of 10; and 2, 1 on each side, external to the central setae and near the marginal setae. Twenty-four setae on ventral body surface, exclusive of those on coxae and anus; 1 near each of 6 coxal plates; 6 anterior to anus arranged in 2 files of 3; and 12 arranged in 2 curved rows of 6 each (inner row occasionally with eight) following curvature of posterior body margin. One seta on each of anal valves. Anal groove completely enclosing anus.

*Scutum.* Five specimens from 0.28 to 0.27 long, average 0.27 and from 0.32 to 0.31 wide, average 0.31. Cervical grooves widely separated, divergent posteriorly almost reaching posterior margin of scutum. Ten setae on scutum; 4 on each side external to cervical grooves and 2 between cervical grooves at about midlength of scutum.

*Legs.* Coxa I with 2 equal, triangular spurs and 3 setae; coxae II and III each with a single, triangular spur and 2 setae. Tarsus I for 5 specimens from 0.21 to 0.20, average 0.20; tapers gradually to anterior end. Haller's organ preceded by shallow sensory pit containing several setae. Twenty setae present on tarsus I.

*Capitulum.* Five specimens, from tip of palpi to posterior margin of basis, from 0.05 to 0.04, average 0.044; width of basis 0.11 for all specimens. Basis triangulate, acute lateral edges and with posterior margin straight. Palpal article 3 longer than article 2 but line of separation not always distinct; lateral margin of article 2 indented and narrowed about 1/3 distance from base; article 4 with several setae; remaining articles with 13 setae, 4 ventrally and 9 dorsally. Basis long ventrally; constricted below midlength; posterior margin rounded. Auriculae as large lateral, triangular projections; margin of basis above auriculae projecting laterally, terminating in point and giving impression of 2 auriculae on each side.

*Hypostome.* Length for 5 specimens from 0.08 to 0.09, average 0.08; dentition 3/3 at anterior third then 2/2 to base; file 1 with 12 denticles, file 2 with 9 and file 3 with 4; lateral denticles large and pointed.

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## Explanation of Plates

Plate I. *Ixodes brunneus* Koch, male: A, scutum; B, capitulum in dorsal view; C, capitulum in ventral view; D, tarsus IV; E, spiracle; F, tarsus I; G, coxa I; H, coxa II; I, coxa III; J. coxa IV.

Plate II. *Ixodes brunneus* Koch, female: A, scutum and body; B, capitulum in dorsal view; C, capitulum in ventral view; D, tarsus I; E, tarsus IV; F. spiracle; G, coxa I; H, coxa II; I, coxa III; J, coxa IV.

Plate III. *Ixodes brunneus* Koch, nymph: A, capitulum in ventral view; B, capitulum in dorsal view; C, spiracle; D, scutum; E, coxa I; F, coxa II; G, coxa III; H, coxa IV.

Plate IV. *Ixodes brunneus* Koch, larva: A, dorsal view; B, ventral view.

PLATE I

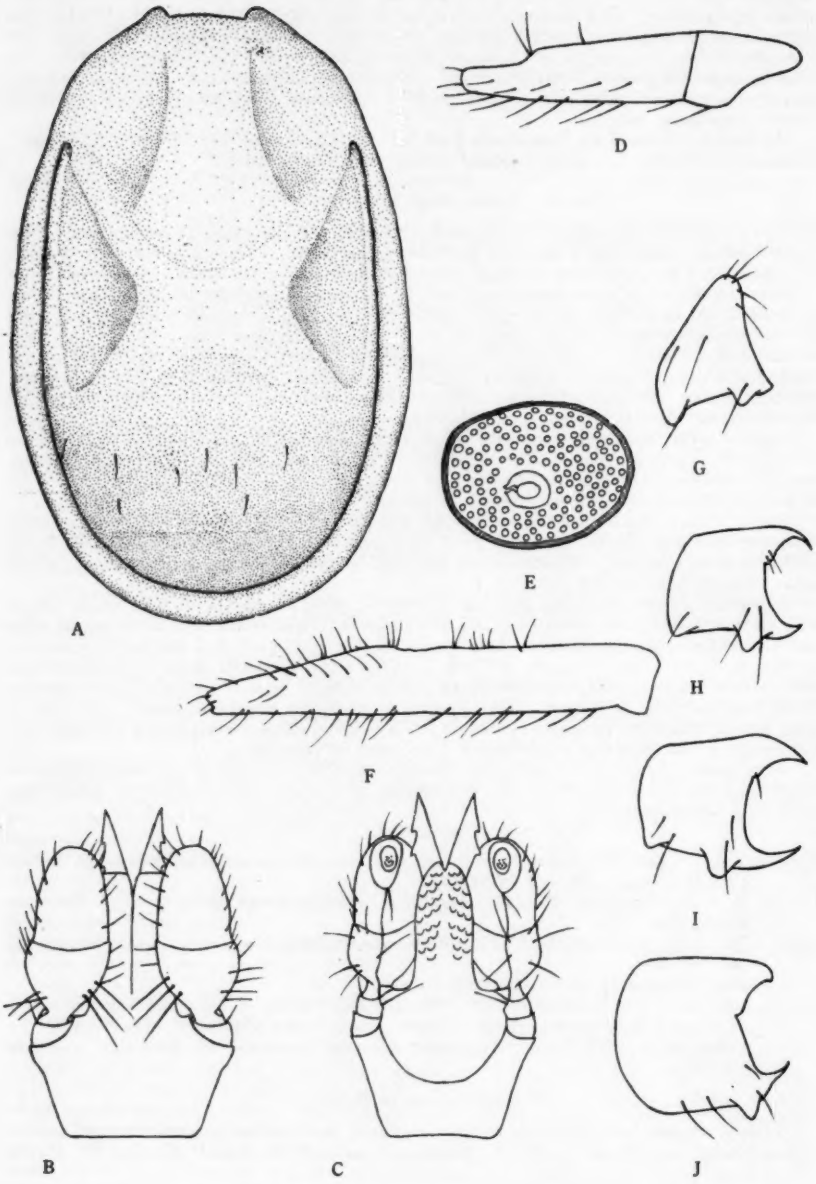


PLATE II

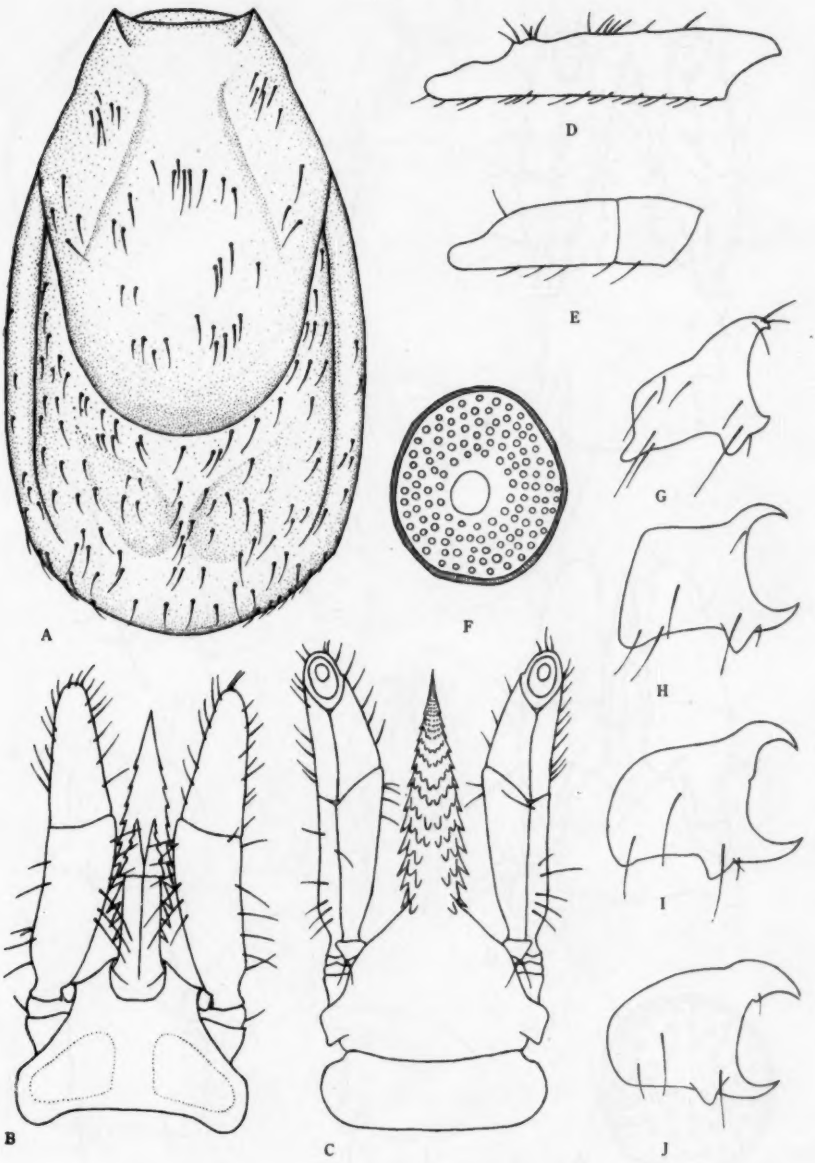
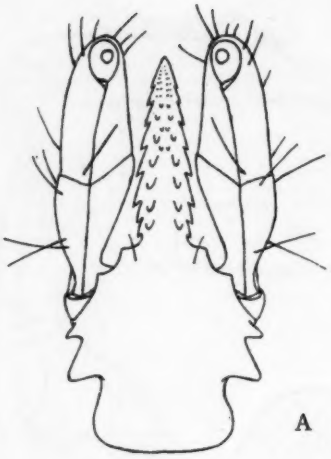


PLATE III



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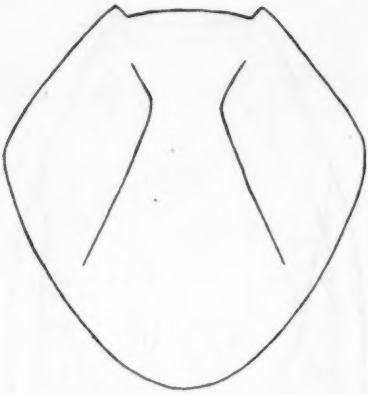

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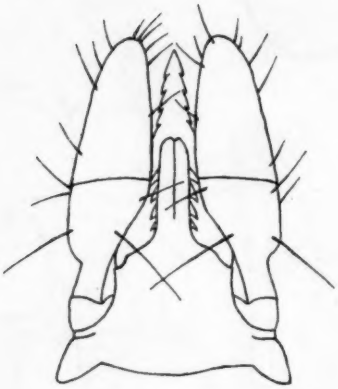

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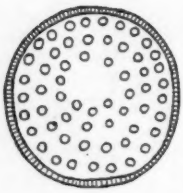

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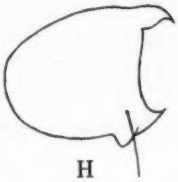
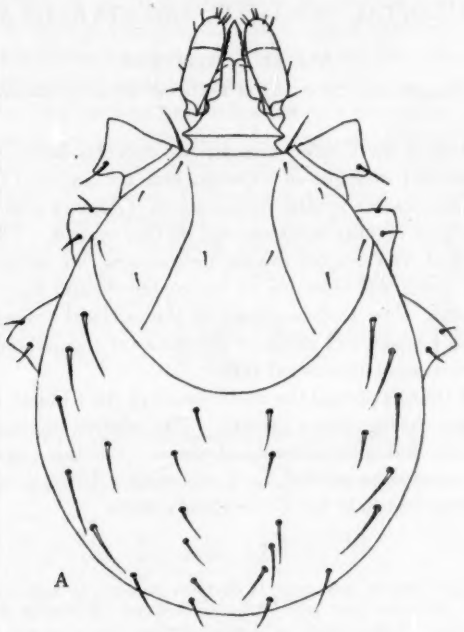


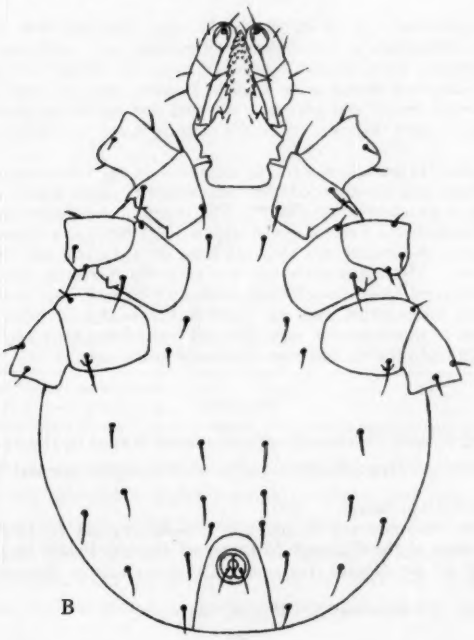
H

G

PLATE IV



A



B

# THE CHINESE HAMSTER (*CRICETULUS GRISEUS*) AS AN EXPERIMENTAL HOST FOR *TRICHINELLA SPIRALIS*

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The cheek pouch of the Golden hamster has recently been used for the *in vivo* observation of vascular changes in experimental trichinosis (Humes and Akers, 1952). Further studies on similar preparations (Altman and Ritterson, unpublished) have disclosed certain shortcomings of this system. The thickness of the everted pouch and of its retractor muscle necessitates, for adequate observation of individual muscle fibers, the resection of the epidermis and much of the inner connective tissue matrix. As a consequence of the surgical procedure, some muscle and vascular damage ensue and much of the retractor muscle remains too thick for satisfactory observation of individual cells.

Fulton *et al* (1954) have used the cheek pouch of the Chinese hamster for studies on vascular changes during tumor growth. The relative thinness of this structure obviates some of the difficulties mentioned above. For this reason, it was decided that the Chinese hamster be studied, on a preliminary basis, to determine its suitability as an experimental host for *Trichinella spiralis*.

## METHODS

Twenty-three adult Chinese hamsters* (*Cricetulus griseus*) of both sexes were fasted for 24 hours preparatory to infection (*per os*). Animals received *Trichinella spiralis*-infected muscle fragments from a donor Golden hamster. Each infective dose consisted of approximately 100 larvae (press count).

Hamsters were sacrificed 3, 5, 14, 26, 40, and 112 days after infection. Employing the Baermann apparatus with physiological saline, the small intestine was examined for the presence of adult worms. Diaphragm press preparations were routinely carried out with an N.I.H.-type trichinoscope. The following tissues were fixed in Bouin's, sectioned and stained with H & E: diaphragm, thigh muscle, heart, and adrenal. Adrenal and spleen weights were taken with a Roller-Smith spring torsion balance. Carcass digests were conducted in the usual way (pepsin-HCl).

Several cheek pouch preparations were accomplished in the following manner (112 days of infection). The animal was anesthetized with nembutal, its cheek pouch everted and fastened in the chamber of an Alex observation dish.** The chamber was filled with Tyrode's solution. Blood vessels and muscle fibers were observed with a Leitz 50× water immersion lens. Following *in vivo* examination, the animal was removed from the apparatus and the pouch pinned to a small, flat slab of cork. The upper epidermis was carefully dissected, exposing the fan-shaped retractor muscle, associated blood vessels, and connective tissue. The pouch was then severed from the hamster and the inverted cork was immediately floated on Bouin's fluid. Following fixation, the hardened retractor muscle was dissected free of the lower epidermis, stained with Ehrlich's hematoxylin, dehydrated, and mounted in the usual manner.

## RESULTS

Adult male and female *Trichinella spiralis* were found in the small intestine from the 3rd to the 26th day after infection. The worms were normal in appearance and

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** Atlantex Corp., 909 Boylston St., Boston, Mass.

within the size range indicated for other experimental hosts (Gould, 1945). Animals sacrificed on days 40 and 112 did not exhibit adult worms. The females removed at 14 and 26 days of infection contained viable first-stage larvae. Some of the latter group were nearly free of larvae. No attempt was made to determine the distribution of worms in the small intestine.

Diaphragm press preparations were taken for each necropsied hamster beginning with the 14th day of infection (Table I). Although most of the diaphragm preparations were negative for larvae, amorphous, granular, spindle-shaped structures were observed in some (Table I). The shape and general appearance suggested that these bodies were poorly formed and/or degenerate cysts in which the worms had undergone dissolution. Histological evidence supported this view (Fig. 1). The possibility of artifact cannot be disavowed. Pepsin digests of large portions of the carcasses confirmed muscle invasion in all cases from the 26th to the 112th day of infection. However, the larval yield appeared meager in terms of the inoculum and persistence of females in the gut.

TABLE I — *Adrenal and spleen-body weight relationships in Chinese hamsters infected with* T. spiralis

| Animal | Sex | Day Post Infection | Body Weight | Adrenal Weight | Adrenal Ratio* | Spleen Weight | Spleen Ratio† | Adults in Gut | Diaphragm Press | Pepsin Digest |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | (gm) | (mg) | | | | | | |
| 1 | F | 3 | 35.5 | 5.8 | 16.3 | 62.6 | 1.76 | Yes | | |
| 2 | F | 3 | 35.0 | 4.2 | 12.0 | 76.4 | 2.18 | " | | |
| 3 | F | 3 | 25.5 | 3.6 | 14.1 | 46.8 | 1.83 | " | | |
| 4 | M | 5 | 47.5 | 12.6 | 26.6 | 72.0 | 1.52 | " | | |
| 5 | M | 5 | 45.8 | 5.5 | 12.0 | 55.4 | 1.21 | " | | |
| 6 | M | 5 | 46.1 | 6.0 | 13.3 | 91.0 | 1.97 | " | | |
| 7 | F | 14 | 30.2 | – | – | 126.6 | 4.21 | " | Negative | |
| 8 | M | 14 | 41.2 | 7.9 | 19.2 | 94.0 | 2.28 | " | " | |
| 9 | M | 14 | 35.7 | 7.0 | 19.6 | 184.4 | 5.17 | " | " | |
| 10 | F | 26 | 33.4 | 6.8 | 20.4 | 92.8 | 2.78 | " | " | Positive |
| 11 | F | 26 | 33.6 | 5.6 | 16.7 | 91.6 | 2.73 | " | ?‡ | " |
| 12 | M | 26 | 42.9 | 5.0 | 11.7 | 159.0 | 3.71 | " | " | " |
| 13 | M | 26 | 31.6 | 5.4 | 17.1 | 102.8 | 3.25 | " | " | " |
| 14 | M | 26 | 35.8 | 4.6 | 12.8 | 56.6 | 1.58 | " | " | " |
| 15 | M | 40 | 51.5 | 6.6 | 12.8 | 78.0 | 1.51 | No | Positive | " |
| 16 | F | 40 | 36.4 | 3.8 | 10.5 | 85.8 | 2.42 | " | " | " |
| 17 | F | 40 | 26.7 | 5.6 | 20.9 | 69.4 | 2.60 | " | ?‡ | " |
| 18 | M | 40 | 42.9 | 5.2 | 12.1 | 56.2 | 1.31 | " | " | " |
| 19 | F | 40 | 26.1 | 4.0 | 15.3 | 77.8 | 2.98 | " | " | " |
| 20 | M | 112 | 37.2 | 5.6 | 15.0 | 34.0 | 0.92 | " | " | " |
| 21 | M | 112 | 43.2 | 7.6 | 17.6 | 46.6 | 1.79 | " | " | " |
| 22 | M | Not | 40.0 | 12.4 | 31.0 | 72.0 | 1.80 | | | |
| 23 | M | infected | 45.7 | 4.0 | 8.8 | 87.4 | 1.91 | | | |

* Expressed as milligrams per 100 grams of body weight.

† Expressed as milligrams per gram of body weight.

‡ Indistinct cyst-shaped structures observed; worms, if present, were not recognizable (degenerate?).

Adrenal weight-body weight ratios (Table I) showed no clear-cut correlation with the status of the infection. Spleen weight-body weight ratios averaged higher during the migratory phase of the infection (Table I). None of the animals exhibited overt signs of the disease and none died during the course of the experiment.

Pathological changes were not observed until the 14th day of infection and were confined to skeletal muscle with 1 or 2 equivocal inflammatory foci in heart muscle

at 26 days of infection. At 14 days, myositis with the characteristic increase and central axis "rowing" of sarcolemmal nuclei was observed. Parasites were not seen in any of the sections. On the 26th day of infection, myositis was extensive with considerable infiltration of leucocytes. Cysts were observed, but none contained morphologically distinguishable larvae (Fig. 1). Infections of 40 days exhibited moderate to severe myositis and nuclear "rowing" in some basophilic muscle fibers. Observed infected foci were not well formed and rarely disclosed what appeared to be degenerate acidophilic fragments of larvae (Fig. 2). At 112 days, muscle changes were confined to infrequent basophilic fibers with nuclear "rowing".

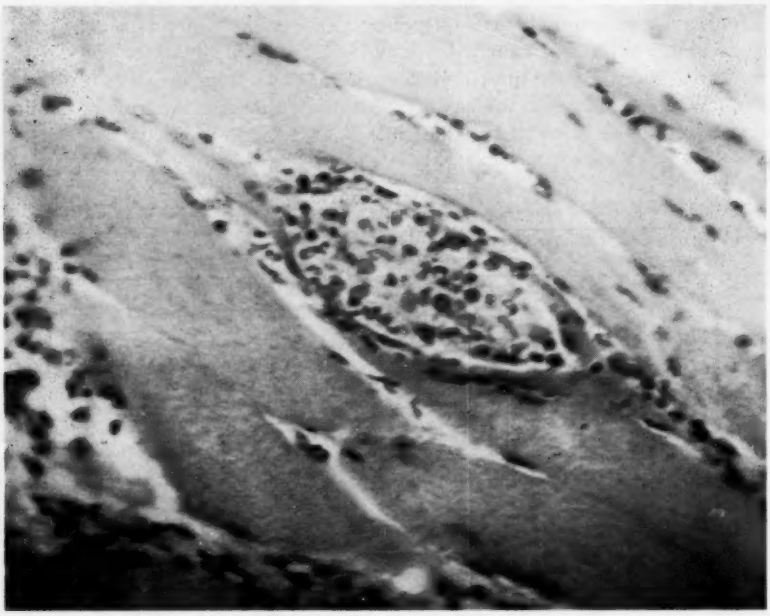

FIGURE 1. Thigh muscle of Chinese hamster after 26 days of infection with *T. spiralis*. Note the absence of a larva in the leucocyte-filled cavity of the cell and the "rowed" nuclei of the invaded fiber at upper right. ×430.

The cheek pouch did not reveal parasites by *in vivo* observation or in stained preparations. Blood circulation was grossly normal and uninfected muscle fibers were clearly seen. Stained specimens exhibited some degree of inflammatory response and a considerable population of fixed tissue histiocytes (Fig. 3).

## DISCUSSION

Although the Chinese hamster has been used for a variety of experimental infections (Yerganian, 1956), *Trichinella spiralis* does not appear to have been reported for this host. The results recorded here indicate its potential value for comparative studies with the closely related Golden hamster. Both animals afford the additional advantage of cheek pouches which permit *in vivo* observation of tissue and circulatory changes.

McCoy (1932) reported that light infections resulted in early elimination of adult trichinae in rats (14 to 16 days). Guinea pigs harbor the parasite up to 55 days and dogs for about 10 days (Gould, 1945). Results reported in this paper indicate an intermediate persistence of worms in the Chinese hamster small intestine. Adults remain in the gut of the Golden hamster for at least 13 days, but animals sacrificed at 26 days revealed no parasites in the intestine (Ritterson, unpublished). Boyd and Huston (1954) found adults in the intestine of the latter host up to the 15th day of infection. Although such data are not yet extensive enough for any of the experimental hosts, it is evident that the Chinese hamster does not exhibit an unusual resistance to the intestinal phase of this parasite.

Diaphragm press preparations (Table I), pepsin digests, and histological specimens (Plate I, Figs. 1, 2) indicate that skeletal muscle is relatively resistant to the permanent lodgement of trichinae. Quantification in terms of the parasite is only possible, and in a rough way, in terms of large groups of animals owing to the variables of dosage (Shaver and Mizzelle, 1955). Viability, longevity, sex ratio, and productivity of females, as well as carcass recovery of larvae, similarly mitigate quantification. However, the relatively small group employed in this investigation was uniformly deficient in muscle larvae in spite of the comparatively long productive life of the adults. Muscle press specimens exhibited worms, some with indistinct cysts, but unfortunately none was observed in tissue sections. Since species differences in muscle composition are known (Spector, 1956), its physiological response to the invading parasite could, in some hosts, result in an unstable environment for the infective agent. Humoral antibody response may be more active in the Chinese hamster. This is not to say that the $LD_{50}$ of *T. spiralis* for the Chinese hamster need differ from that of the Golden hamster, as myositis was extensive in the former host. Such possibilities await further study for their proper assessment.

Variations in adrenal weight-body weight ratios were considerable and appeared to be unrelated to the experimental infection. Spleen weight-body weight ratios averaged higher during the invasive stage, i.e., 14 to 26 days (Table I). It is probble that spleen ratios at 3, 5, and 112 days of infection correspond to normal values. However, the small number of animals employed in this study requires that alternate explanations be considered. Intermittent enteric infections which may become chronic are common among laboratory animals (Griffith and Farris, 1942) and may account, in part, for the observed fluctuations in organ weights. The normal Golden hamster does not appear to exhibit much variation in adrenal or spleen ratios (Ritterson, 1955).

The comparative value of the Chinese hamster is enhanced by the presence of the cheek pouch. Although parasites were not observed in the specimens examined, its usefulness has been demonstrated for the Golden hamster (Humes and Akers, 1952). Stained retractor muscle preparations of the uninfected Golden hamster have been studied by the author. The specimens indicate that there is a normal transient inflammatory response of the pouch and that pathodynamic changes in experimental infections should be interpreted with due caution. The absence of inflammatory response to tissue sections at 112 days of infection indicates that the cellular picture in the cheek pouch corresponds with that found in the normal Golden hamster.

Though quantitative evaluation of parasite burdens is lacking, the data justify

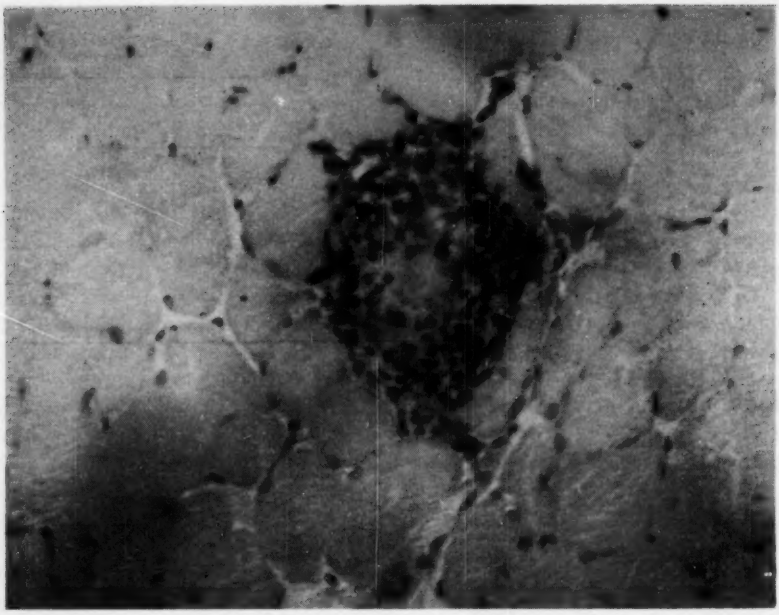

FIGURE 2. Thigh muscle of Chinese hamster after 40 days of infection with *T. spiralis*. Note inflammatory cells surrounding the acidophilic central mass. ×430.

FIGURE 3. Cheek pouch of Chinese hamster. Whole mount after 112 days of infection with *T. spiralis*. Individual muscle fibers may be seen with densely stained histiocytes on their surfaces. Note blood vessels and moderate leucocytic response in vicinity of the fibers. ×430.

the tentative conclusion that the Chinese hamster provides a less suitable environment for the muscle phase of *T. spiralis* than does the Golden hamster. The reversed situation obtains with respect to the intestinal phase. The Chinese hamster is known to be considerably more resistant to kala-azar (Meleney, 1925) which is uniformly fatal to the Golden hamster. The well-known effects of cortisone on the inflammatory response should be of value in the further clasification of the nature of the apparent resistance of the Chinese hamster to *T. spiralis*.

The author has recently established a colony of Chinese hamsters through the courtesy of Dr. George Yerganian and further comparative studies are in progress.

## SUMMARY

Each of 21 Chinese hamsters was infected with approximately 100 larvae of *T. spiralis* from a Golden hamster. Adult worms were recovered from the small intestine through the 26th day of infection. Small numbers of viable larvae were recovered from carcasses of hamsters infected for 26 days or more.

Adrenal weight-body weight ratios were highly variable and had no clear relation to the experimental infection. Spleen-body weight ratios averaged higher during the migratory phase of the infection.

Histological exmaination of the adrenal exhibited no pathological changes. Myositis was evident at 14 days of infection. At 26 and 40 days, the inflammatory reaction was moderate to severe in all animals. Cysts were observed in sections, but none showed normal larvae. Diaphragm press and/or pepsin digests confirmed infection in all animals with infections of 26 or more days' duration. Minimal muscle reaction was observed at 112 days.

*In vivo* observations on cheek pouches at 112 days revealed no parasites. Stained whole mounts of retractor muscle disclosed an inflammatory response which appeared not to be related to the experimental infection.

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# STUDIES ON OVINE COCCIDIOSIS. I. SOME PHYSIOLOGICAL CHANGES TAKING PLACE IN EXPERIMENTAL INFECTIONS WITH *EIMERIA NINAE-KOHL-YAKIMOVI* (YAKIMOV AND RASTEGAEVA, 1930) AND *EIMERIA FAUREI* (MOUSSU AND MAROTEL, 1901)

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Studies concerning physiological effects of coccidia on their hosts have been chiefly confined to *Eimeria tenella* and the domestic fowl. The purpose of this study was to determine the effect of coccidial infections on levels of inorganic serum phosphorus, serum protein, blood glucose, blood hemoglobin, total volume of red cells, and on the digestibility of feed protein in lambs. Unfortunately, a pure culture of *Eimeria ninae-kohl-yakimovi* oocysts was not available, therefore a mixed infection of this species and *Eimeria faurei* was used.

## MATERIALS AND METHODS

Eight grade lambs of approximately the same age (50 days) raised "parasite free" by a method devised by Shumard and Eveleth (1956), were placed in 2 pens so that 3 wethers and 1 ewe were in the first lot and 2 wethers and 1 ewe in the second. Pens were of concrete construction with a central drain in each. No bedding was used but each pen was cleaned by washing daily.

The ration was a pelleted feed consisting of 40% green alfalfa meal, 49% ground yellow corn, 10% molasses and 1 % trace mineralized salt. Before pelleting, 10 pounds of chromium oxide was uniformly mixed with each ton of feed as a base for determining protein digestibility. Daily feed and water consumptions were recorded. As each bag of feed was opened for use, an aliquot of the feed was analyzed for protein and chromium oxide. Aliquots of fecal material from the wethers were analyzed for protein and chromium oxide. Analyses for protein were accomplished by a modified Kjeldahl method, using selenium as a catalyst. Chromium oxide determinations were according to Bolin *et al* (1952).

All animals were weighed at approximately weekly intervals. Two blood samples of 5 ml each were withdrawn from the jugular vein of each lamb every other day. One sample was allowed to clot, the other was mixed with sodium citrate (0.05 ml of a 10% solution, evaporated to dryness). The serum was removed from the clotted samples and analyzed for inorganic phosphorus (modified from Fiske and Subbarow, 1923), total protein (Kingsley, 1939) and albumin (Pillemer and Hutchinson, 1945). Serum globulin was calculated. Hematocrit values were determined by the method of Van Allen (1925), blood glucose by a modification of the Folin-Wu method (1919), and hemoglobin by the oxyhemoglobin method.

Each of the lambs in 1 lot was given approximately 7,000,000 *Eimeria ninae-kohl-yakimovi* and 100,000 *Eimeria faurei* sporulated oocysts by means of a drenching syringe. Fecal flotations were made daily. As soon as oocysts appeared, protein digestion analyses were concluded because of the chance of error due to the presence of oocysts.

## RESULTS

Clinical signs of coccidiosis appeared in the form of lowered feed consumption on the 9th day following infection and in general lassitude, blanching of the skin and

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The author is grateful for the advice and assistance of D. W. Bolin and the technical assistance of D. J. Hanson, C. Lohse and F. Hesse. He also wishes to express his thanks to Dr. John C. Lotze, A.R.S., U.S.D.A. for supplying the oocysts used in this investigation.

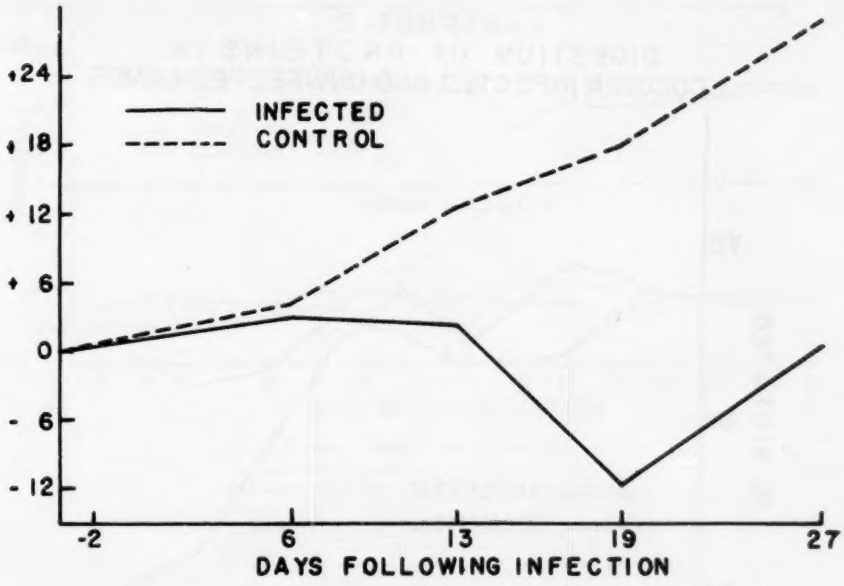
a generalized incoordination especially in the forequarters and head region beginning on the 12th day. A typical attitude was a prone position; the lamb lying fully on its undersurface with the forelegs in an awkward unparallel position to the front, and the head bent so far to the left that the lamb was looking at its hind quarters. From the 13th to the 20th day, an uncoordinated nodding of the head was noted. This occurred to a greater degree during the last 48 hours of morbidity in the one lamb that died. Fecal consistency became light in the 3 surviving lambs beginning on the 15th day. The lambs scoured for a few days but persistent diarrhea was not present. At no time was extensive bleeding noted although tinges of partially digested blood appeared in the feces at intervals following the 15th day.

One lamb died on the 15th day. Necropsy within 10 minutes following death revealed thickened and edematous mesenteries. The walls of the jejunum and ileum were thickened. Small intestinal contents were fluid and streaked with blood and mucous. The cecum contained semifluid material flecked with blood and mucous.

Feed and water consumptions of the infected lambs began to decrease on the 9th day following infection, resulting in weight loss as depicted in Figure 1 and Table I.

FIGURE 1

PERCENTAGE CHANGE OF TOTAL WEIGHTS OF COCCIDIA INFECTED AND UNINFECTED LAMBS




The resumption of a more normal feed consumption beginning on the 22nd day resulted in weight recovery without overall gain at 27 days following infection. Even though total water consumption decreased, the amount of water taken in per pound of body weight did not fluctuate significantly while the amount of feed con-

TABLE I—*Average daily feed and water consumed per pound of infected and control lamb**

| Days Following Infection | Feed lbs. | | Water qts. | |
|---|---|---|---|---|
| | Infected | Control | Infected | Control |
| 0-5 | .018 | .020 | .022 | .021 |
| 5-10 | .017 | .019 | .025 | .023 |
| 10-15 | .011 | .020 | .022 | .024 |
| 15-20 | .011 | .025 | .021 | .025 |
| 20-25 | .013 | .025 | .020 | .024 |
| 25-27 | .015 | .022 | .021 | .024 |

* Based on average weight for period indicated and total feed and water consumed during the period.

sumed per pound of body weight decreased as much as 38% (Table II). This is important from the standpoint of therapeusis.

The digestion of protein available in the feed began to decrease in amount on about the 9th day in the infected lambs and continued to decrease until the analyses were discontinued (Figure 2 and Table III). Fluctuations that occurred in the control lambs fell within the standard error. Total serum protein did not fluctuate significantly during the experiment; serum albumin, however, decreased on about the 15th day while a corresponding increase in serum globulin occurred. These serum protein changes, as depicted in Figure 3, represent a decrease in the albumin/

FIGURE 2
DIGESTION OF PROTEINS IN
COCCIDIA INFECTED AND UNINFECTED LAMBS

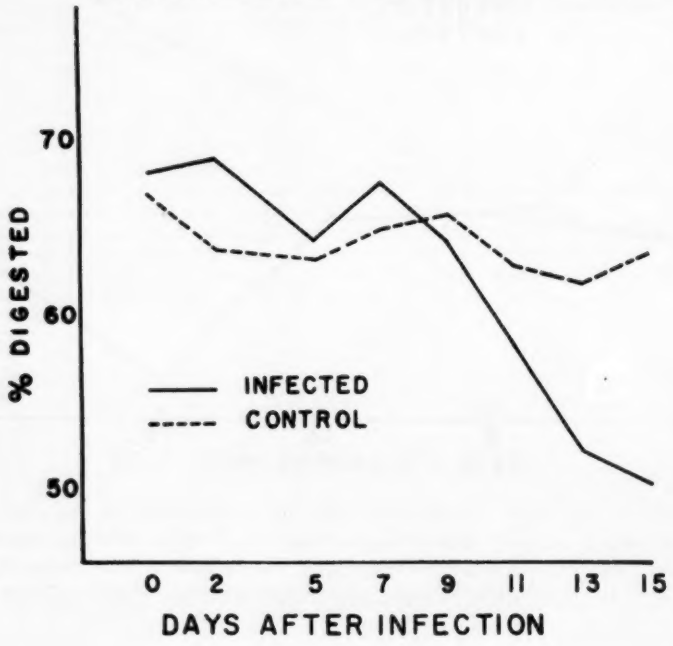


TABLE II — *Weights of coccidia infected and uninfected lambs — in pounds*

| Days Following Infection | Infected | | | | Uninfected | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| -2 | 46 | 45 | 38 | 38 | 36 | 52 | 38 | 35 |
| 6 | 45 | 48 | 38 | 37 | 38 | 53 | 40 | 37 |
| 13 | 47 | 50 | 37 | 37 | 39 | 61 | 43 | 38 |
| 19 | 46 | 46 | – | 37 | 41 | 65 | 45 | 39 |
| 27 | 54 | 52 | – | 41 | 45 | 72 | 49 | 41 |

globulin ratio from a normal value of about 1.6 up to and including the 13th day to a low of 0.8 on the 15th day.

FIGURE 3

TOTAL SERUM PROTEIN, SERUM ALBUMIN AND SERUM GLOBULIN IN COCCIDIA INFECTED AND UNINFECTED LAMBS

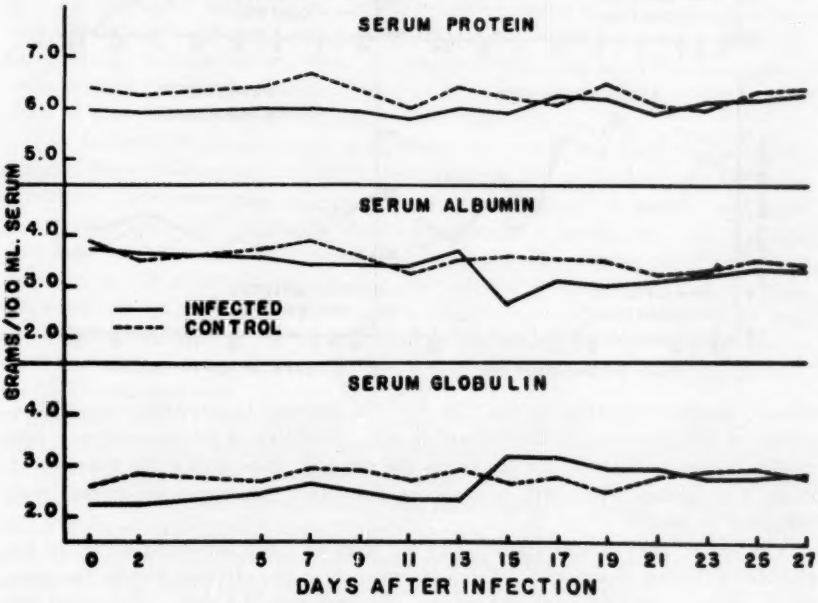
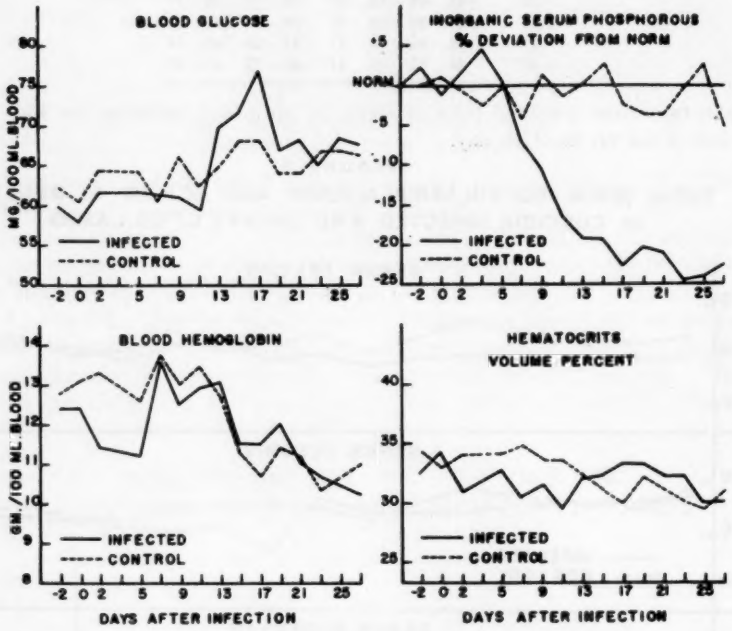



TABLE III — *Percentage of feed protein digested by coccidia infected and uninfected wethers*

| Days Following Infection | Infected | | | Uninfected | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 |
| 0 | 69.85 | 66.96 | 69.77 | 69.77 | 60.94 |
| 2 | 65.11 | 72.37 | 70.30 | 64.37 | 58.96 |
| 5 | 62.07 | 69.40 | 62.22 | 65.11 | 56.38 |
| 7 | 64.22 | 76.51 | 62.59 | 66.96 | 62.09 |
| 9 | 63.78 | 74.59 | 55.63 | 68.37 | 61.44 |
| 11 | 60.44 | 58.37 | 56.22 | 63.78 | 59.37 |
| 13 | 62.51 | 46.51 | 49.19 | 64.82 | 56.76 |
| 15 | 58.96 | 42.70 | – | 66.16 | 55.94 |

Levels of blood glucose, inorganic serum phosphorus, blood hemoglobin and hematocrits are graphed in Figure 4. A rise in blood glucose occurred among the

FIGURE 4
BLOOD GLUCOSE, INORGANIC SERUM PHOSPHOROUS, BLOOD HEMOGLOBIN LEVELS AND HEMATOCRIT VALUES OF COCCIDIA INFECTED AND UNINFECTED LAMBS




infected animals beginning on the 11th day. A decrease in inorganic serum phosphorus of 25% occurred in the infected lambs. Recovery of this element to a previously determined normal for the lambs did not take place during the experiment. Blood hemoglobin levels and hematocrits fluctuated but remained above levels indicative of anemia.

Oocysts of both species appeared in the feces of the 3 surviving lambs on the 15th day following inoculation. From observation in lieu of direct counts the number of oocysts produced increased between the 16th and 21st days. Following this a gradual decline in production took place although all 3 lambs were passing oocysts of both species at the termination of the study.

## DISCUSSION

Antemortem manifestations of coccidiosis produced by *Eimeria ninae-kohlyakimovi*, as described by Lotze (1953), were observed to take place. The presence of symptoms indicative of nervous disorder cannot, at the present time, be ascribed to a known pathological entity, although incoordination is a classical symptom occurring during infections with *E. tenella* in chickens. This latter observation, however, may well be due to the severe hemorrhage which normally

occurs. Other physiological changes taking place, suggestive of nervous inhibition, during infection with *E. tenella* were reported by Levine and Herrick (1954, 1955) and Schildt and Herrick (1955).

Increases in blood sugar in chickens infected with *E. tenella* have been described by Herrick (1933, 1939), Pratt (1940), and Waxler (1941). The latter investigator observed that the same phenomenon occurred when phlebotomy was used to simulate hemorrhage. Pratt (1941) attributed the increase to formation of glucose from muscle lactic acid. Daugherty and Herrick (1952) presented a hypothesis that the accumulation of blood glucose during infection was in part due to an interference with normal phosphorylation. In the present investigation, hemoglobin and hematocrit values remained within a range that was not indicative of even moderate hemorrhage, much less anemia. While blood glucose levels of the infected lambs did not rise to the extent as that described by Pratt (1940) in chickens, a marked departure from previous levels and those of the controls taken at the same time occurred during the time when second generation schizogony and sporogony were taking place. While no experimental evidence can be offered to explain this hyperglycemia, it is possible that stress, produced during coccidiosis, stimulates an increase in secretion of glucocorticoid hormone from the adrenal cortex, resulting in the production of glucose from muscular sources, while an inhibition of phosphorylation of available glucose takes place.

The decline in inorganic serum phosphorus occurred at the same time feed consumption began to drop and paralleled the development of first generation schizonts. It became more pronounced as second generation schizonts developed and sporogony progressed. The tendency toward recovery to a normal level did not take place until oocyst-production dropped, despite a return to original weight and feed consumption. It is possible that during the endogenous development of the parasite, relatively large amounts of inorganic phosphorous are required. This may account for the decrease of this substance in the serum when additional sources are not available due to a decrease in feed consumption and the inability of the host to compensate otherwise.

Herrick (1933, 1939) described decreases of total and non-protein nitrogen that occurred during hemorrhage due to infections with *E. tenella*. Bertke and Herrick (1954) observed a decrease in uric acid excretion to 64% on the 4th day and to 46% on the 10th day following inoculation with *E. tenella*. These pathological changes could have resulted from a change in blood volume and lack of an adequate fluid retention due to severe hemorrhage. However, uric acid excretion, as described by the authors, did not return to previous levels by the 3rd week after infection. This would suggest responsible factors other than hemorrhage. In the present investigation no significant changes in total serum protein took place during the infection. However, a decrease in albumin and a corresponding increase in globulin of about 25% occurred 15 days following inoculation of the lambs with oocysts of the two species. While no experimental evidence is at present available, the relative increase in globulins may possibly have been due to an increase in the gamma fraction.

The loss of efficiency in digesting proteins available in the feed began at a time when increased growth and development of the endogenous stages of the parasites was accelerating. Whether the decrease in digestion was due to loss of enzymatic

efficiency, failure of proper absorptive processes, lack of proper rumen function, or other processes is not known.


## SUMMARY

Lambs infected with the coccidia *Eimeria ninae-kohl-yakimovi* and *Eimeria faurei* exhibited clinical symptoms of lowered feed consumption, lassitude, generalized incoordination and slight scouring with some bleeding. No significant decrease in water consumption per pound of lamb took place.

A decrease in the percentage of feed protein digested occurred. Total serum protein did not fluctuate but changes in the albumin/globulin ratio indicated an increase in globulins. While blood hemoglobin and hematocrit values indicated a lack of even moderate hemorrhage, inorganic serum phosphorus levels dropped and blood glucose levels rose.

All of the physiological changes that took place in the host could be correlated with the growth and development of the endogenous phases in the life cycle of the coccidia.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# *SYPHACIA MURIS* VS. *S. OBVELATA* IN LABORATORY RATS AND MICE

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Oxyurid nematodes of the genus *Syphacia* are commonly found in the cecum of albino rats and mice, some colonies having a very high infection rate. Interest in these worms has increased recently because of their use as test organisms in chemotherapeutic studies on enterobiasis. For several years we have been carrying on such studies using the 2 oxyurids of mice, *Aspiculuris tetraptera* and *Syphacia obvelata*. We noted large numbers of oxyurids from the cecum and occasionally the first part of the large intestine of rats from Carworth Farms. Male worms had the 3 ventral cuticular projections ("mamelons") characteristic of the genus *Syphacia*, while gravid females contained eggs with a flattened side. However, these eggs from specimens of *Syphacia* from the rat were distinctly different from the eggs of worms of the same genus from the mouse (Figs. 2, 3). Further examination of adult specimens from the rat showed some additional differences:

1. Worms of both sexes from the rat are slightly smaller than mouse forms.
2. Eggs of the rat worm are only a little more than ½ the length of those from worms from the mouse, although nearly the same width.
3. Position of the excretory pore in both sexes and vulva of the female is further posterior (both actually and relatively) to the esophageal bulb than in the mouse species.
4. Tail of the male is thinner with length approximately twice the body width, while in the mouse species the tail length is approximately equal to the body width.
5. Anterior mamelon of the male is close to the middle of the body, while in the mouse species the middle mamelon is close to the middle of the body.

These differences are shown in Figures 1–4 and are confirmed by measurements (average of 20 worms) (Table I).

Yorke and Maplestone's (1926) figures of the adult male and the egg of *S. obvelata* correspond to those of worms we found in the rat but not in the mouse, while Chitwood and Chitwood's (1950) figure of the egg of *S. obvelata* corresponds to those of worms we found in the mouse. It appears that specimens from both mice and rats were lumped together in the species *S. obvelata* (Rud., 1802) Seurat, 1916 by Yorke and Maplestone (1926) when they redescribed the genus, giving *S. obvelata* as type species and mice, rats and man as hosts.

Yamaguti (1935) originally described the oxyurid from the albino rat as *Enterobius muris*, on the basis of females only. After obtaining males, he emended this description (1941) and transferred it to the genus *Syphacia*.

Our mouse species corresponds to Seurat's (1916) description of *S. obvelata* (Rud., 1802) while the rat specimens correspond to Yamaguti's (1941) descrip-

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tion of *S. muris,* although appearing to be more mature, as we found gravid females to contain eggs with well developed larvae.

The various described species of *Syphacia*** may be divided into two groups according to whether the male has 2 or 3 mamelons, as Tiner (1948) has done in his key to species of the genus. Recently, Chabaud and Biocca (1955) have similarly divided the genus into 2 subgenera, *Syphatineria* (2 mamelons in the males; parasitic in Sciurinae) and *Syphacia* (3 mamelons in the males; parasitic in Muridae and rarely other rodents). However, their differentiation of subgenera on the basis of host family does not appear to be valid, as at least 4 of the 9 species of the 3-mamelon group of the genus *Syphacia* are described from hosts belonging to families other than Muridae. Roman originally (1945) described *S. ratti* (from female specimens only) from *Rattus rattus* and later (1951) concluded that this and *S. bay-*

TABLE I — *Comparative measurements of* Syphacia obvelata *and* Syphacia muris

| | Females | | Males | |
|---|---|---|---|---|
| | *S. obvelata* | *S. muris* | *S. obvelata* | *S. muris* |
| Total length | 5.203 mm | 4.018 mm | 1.334 mm | 1.234 mm |
| Greatest width | .312 | .252 | .131 | .112 |
| Ant. tip - excretory pore | .523 | .558 | .302 | .357 |
| Ant. tip - vulva | .874 | .962 | | |
| Tail length (anus - tip) | .762 | .718 | .122 | .229 |
| Ant. tip - ant. mamelon | | | .470 | .575 |
| Ant. tip - mid. mamelon | | | .618 | .684 |
| Egg length | .134 | .075 | | |
| Egg width | .036 | .029 | | |
| Ant. tip - excretory pore / Total length | 1/9.9 | 1/7.2 | 1/4.4 | 1/3.5 |
| Ant. tip - vulva / Total length | 1/6.0 | 1/4.2 | | |
| Ant. tip - ant. mamelon / Total length | | | 0.35 | 0.45 |
| Ant. tip - mid. mamelon / Total length | | | 0.46 | 0.55 |
| Tail length / Greatest width | | | 0.9 | 2.0 |

*lisi* Maplestone and Bhaduri 1942 (also from female specimens only) were both synonyms of *S. muris* (Yamaguti, 1941), since all were very similar and were found in *Rattus* sp.

Adding *S. arctica* (Tiner and Rausch, 1950) to those included in Tiner's (1948) key gives 8 described species of *Syphacia* in which the males have 3 mamelons. Tiner (1948) suggests that *S. venteli* (from *Nectomys squamipes* Brants), although from a different host genus, is also a synonym of *S. muris.* Pending further study, this then would leave 7 species of the 3-mamelon group of the genus *Syphacia—S. obvelata, S. muris, S. stroma, S. thompsoni, S. peromysci, S. arctica* and *S. nigeriana.* Of these, *S. thompsoni, S. peromysci, S. arctica* and *S. obvelata*

** Specimens of *Syphacia* from the albino rat and mouse were submitted to the Animal Disease and Parasite Research Branch, Agricultural Research Center, U.S.D.A., where they were examined by Mrs. M. B. Chitwood. They are listed as U.S.N.M. Coll. #41197 (*S. muris*) and #41196 (*S. obvelata*).

are described from North America. Figures 5–11 show the male tails of these 4 species and *S. muris,* all drawn to the same scale.

Since *S. muris* had not been described from North America we wished to determine whether it was of local or widespread occurrence.

Correspondence with a number of parasitologists showed that many had noted the differences in the eggs of the species from albino rats and mice. Tiner (personal communication) stated that while at the Merck Institute for Therapeutic Research, he had identified oxyurids from Carworth Farms rats as *S. muris.* Manter (personal communication) noted that a nematode from rats which at first was thought to be *S. obvelata* was identified as *S. muris* by Mr. H. E. Heidtke in 1951 and that the paper by Prince (1950) actually pertained to *S. muris.*

As the worms deposit their characteristic eggs in the perianal region, infection may be detected by the use of anal swabs as for enterobiasis in man. An examination of swabs from several groups of rats and mice kept in separate rooms in the Department of Animal Care, as well as from another colony at the College of Physicians and Surgeons, Columbia University, showed the presence of *S. obvelata* in the mice and *S. muris* in the rats.

Through the kindness of colleagues we were able to obtain anal swabs and/or preserved worms from rat and mouse colonies in various parts of the United States and one locality in Canada. From the summary of these results (Table II) in all localities surveyed, *S. obvelata* appears to be the common *Syphacia*

TABLE II — *Distribution of* Syphacia *sp. in laboratory rats and mice*

| Source | | Rats | Mice |
|---|---|---|---|
| New York, N.Y | Dr. C. A. Slanecz, Columbia Univ. | 11/12 *S. muris*; 7/15 *S. muris* | 9/19 *S. obvelata* |
| | Dr. R. Rugh, Columbia Univ. | 3/4 *S. muris* | |
| | Miss N. Alger, N.Y.U. College Med. | | 15/27 *S. obvelata*; 2/27 *S. muris*†‡ |
| Boston, Mass. | Dr. A. G. Humes, Boston Univ. | 5/6 *S. muris** | |
| Storrs, Conn. | Mr. A. Wagner, Univ. Conn. | 4/16 *S. muris* | 6/9 *S. obvelata* |
| Urbana, Ill. | Dr. N. D. Levine, Univ. Ill. | 1/1 *S. muris* | 5/6 *S. obvelata* |
| Phila., Pa. | Dr. I. Kagan, Univ. Pa. | | 3/7 *S. obvelata* |
| Ann Arbor, Mich. | Dr. R. J. Porter, Univ. Mich. | 8/10 *S. muris* | 15/24 *S. obvelata** |
| Detroit, Mich. | Mrs. A. R. Cook, Parke-Davis | | § *S. obvelata** |
| Columbia, Mo. | Dr. R. Campbell, Univ. Missouri | 2/3 *S. muris*; 1/3 *S. obvelata*†‡ | |
| Chapel Hill, N.C. | Mr. R. H. Sudds, Jr. Univ. N.C. Sch. P.H. | | § *S. obvelata** |
| Chamblee, Ga. | Mr. A. J. Sulzer, C. D. C. | | 6/15 *S. obvelata*; 4/15 *S. muris*?†‡ 2/15 doubles? †‡ |
| Dallas, Texas | Dr. D. V. Moore, Univ. Texas | | 1/15 *S. muris*;† 9/15 *S. obvelata* |
| Houston, Texas | Mr. J. C. Holmes, Rice Institute | § *S. muris** | § *S. obvelata** |
| Tallahassee, Fla. | Dr. R. Short, Florida State Univ. | | § *S. obvelata** |
| Dugway, Utah | Mr. S. R. Geiger, Dugway Proving Ground | | 4/4 *S. obvelata*; § *S. obvelata** |
| Toronto, Ontario Canada | Dr. R. S. Freeman | 7/8 *S. muris** | 8/8 *S. obvelata** |
| | Ontario Research Foundation | 5/5 *S. muris** | |

From Scotch tape anal swabs except where noted otherwise.
Figures given = number positive/number animals examined.
* Preserved worms.
† Cross infection?
‡ Mice and rats kept near together.
§ Number of animals not indicated.

of laboratory mice and *S. muris* the common *Syphacia* of laboratory rats, although the mouse may occasionally serve as the host for *S. muris* and the rat for *S. obvelata.* Of the 4 instances of cross infection, 3 were in colonies in which rats and mice were kept in the same room. Cross infection occurred in our laboratory when mice with experimental *S. obvelata* infection were kept in the same room with rats with experimental *S. muris* infection.

We also demonstrated cross infection experimentally by giving nematode-free mice naturally shed eggs of *S. muris.* Positive anal swabs were obtained, and gravid female *S. muris* then recovered from the mice.

SUMMARY

1. *S. muris* may be most easily differentiated from *S. obvelata* by size of the egg, positions of excretory pore and vulva of female, and position of excretory pore and mamelons and length of tail of mature male.

2. *S. obvelata* is the usual *Syphacia* of albino mice.

3. *S. muris* is the usual *Syphacia* of albino rats.

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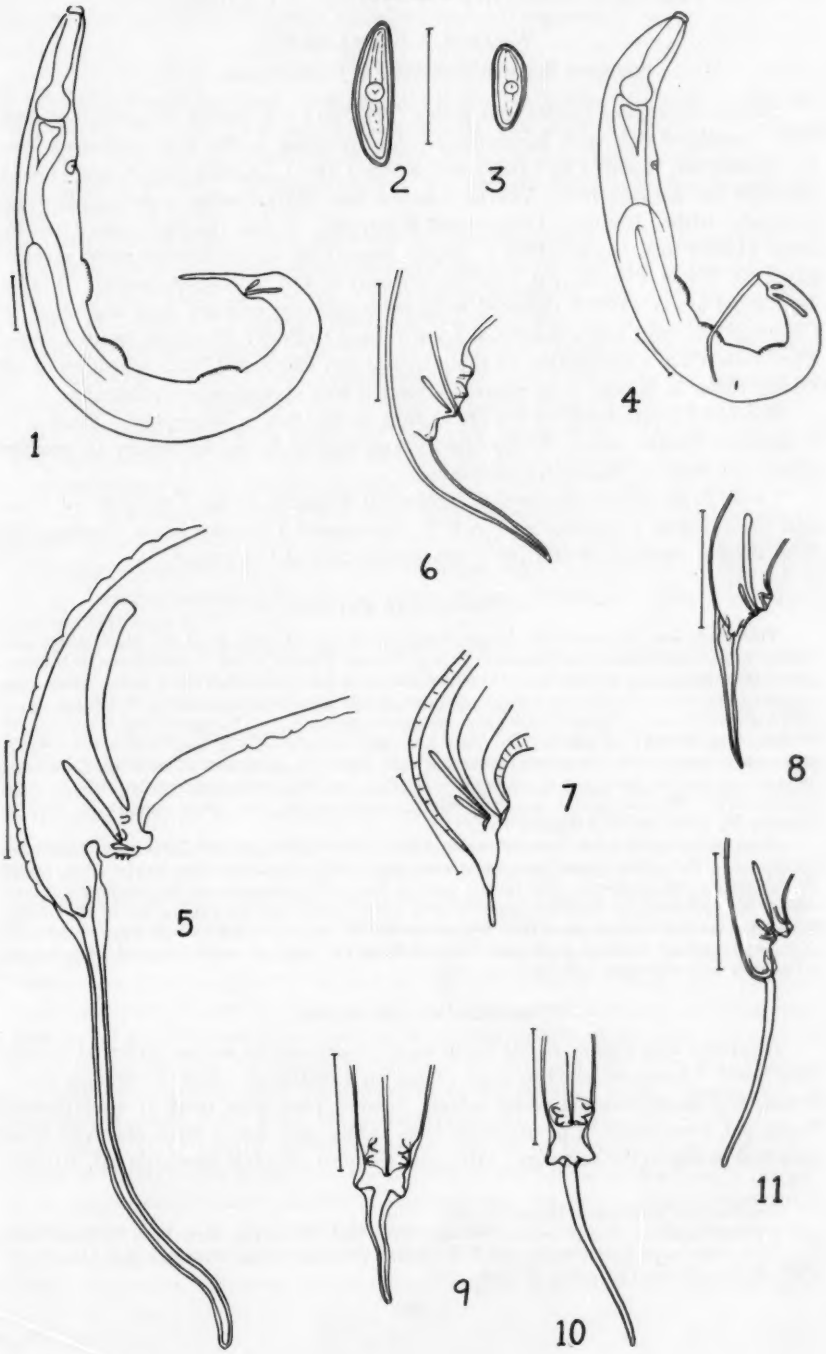
## Explanation of Plate

(Figures 1–4, 8–11 drawn with the aid of the camera lucida. Scale for all figures—100 microns.)

Figure 1. *Syphacia obvelata* male
Figure 2. *S. obvelata* egg
Figure 3. *S. muris* egg
Figure 4. *S. muris* male
Figure 5. *S. thompsoni* male tail—redrawn after Price 1928
Figure 6. *S. arctica* male tail—redrawn after Tiner and Rausch 1950
Figure 7. *S. peromysci* male tail—redrawn after Harkema 1936
Figure 8. *S. obvelata* male tail—lateral view
Figure 9. *S. obvelata* male tail—ventral view
Figure 10. *S. muris* male tail—ventral view
Figure 11. *S. muris* male tail—lateral view

PLATE I



# THE LIFE HISTORY OF *OTOBIUS LAGOPHILUS*

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*Otobius lagophilus* Cooley and Kohls, 1940, is a soft-bodied tick which is normally associated only with lagomorphs. An exception to this host preference was an infested cat, reported by Cooley and Kohls (1944). Cooley and Kohls (1944) recorded the parasite from Alberta, Canada, and the following states: California, Colorado, Idaho, Montana, Oregon and Wyoming. It was reported from Utah by Beck (1955) and Hopla (1955). Some aspects of its life-history were investigated by Hopla (1955), *e.g.* type of oviposition and incubation period. Philip, Bell and Larson (1955) reported it to be apparently infected with the virus of Colorado tick fever and a rickettsial agent related to Rocky Mountain spotted fever. They referred to a publication by Silva-Goytia and Elizondo (1952) which reported *O. lagophilus* in Mexico to be naturally infected with spotted fever rickettsiae.

Published information on the life history of this tick is incomplete. Since it is a potential disease vector, its life history was studied in the laboratory to provide a basis for study of disease transmission.

I wish to thank the members of Ecological Research of the University of Utah and the editorial committee of the BW Assessment Laboratories at Dugway for their helpful suggestions relative to the preparation of this report.

## MATERIALS AND METHODS

This study was initiated with nymphs obtained March 15, 1955 from the black-tailed jack rabbit, *Lepus californicus,* in Dugway Valley, Tooele County, Utah. Subsequent collections were made throughout the spring and summer whenever infested rabbits were found. Engorged nymphs were removed from the rabbits with eye-muscle forceps and placed in 17×60 mm glass shell vials which were stoppered with gauze-wrapped cotton plugs. This type of vial was also used to store eggs, larvae, and adults. The vials were laid on their sides in open 6-inch Petri dishes which were then stored in closed, desiccator-type jars. A saturated solution of ammonium sulfate was used in each jar to maintain a relative humidity of approximately 81% at room temperature. The average of the daily median room temperature from August 26, 1955 to January 18, 1956, was 76.7 degrees F.

Engorged nymphs taken from jack rabbits shot in the field molted to adults under laboratory conditions. The adults mated and eggs were laid. After hatching, the larvae were placed on rabbits for engorgement. The rabbits used in the study were infested by brushing approximately 200 to 300 larvae from the gauze-covered cotton plugs onto the hair on top of the rabbit's head. Larvae and nymphs were later found attached on the face, head and the base of the ears. Nymphs remained attached until they dropped from the host, or were removed with forceps when they had engorged sufficiently to molt.

## RESULTS AND DISCUSSION

A captive jack rabbit, *Lepus californicus,* was found to be the preferred laboratory host. Larvae attached to some extent on a cottontail rabbit, *Sylvilagus audubonni,* and on a domestic white rabbit. Guinea pigs were tried as experimental hosts, but were found to be unsatisfactory. Only once was a larva observed to be attached to one of the test pigs. Attempts to infest an adult kangaroo rat, *Dipodo-*

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*mys ordii,* a suckling deer mouse, *Peromyscus maniculatus,* and a suckling western harvest mouse, *Reithrodontomys megalotis* were all negative.

### *Nymphal Stage*

As determined by direct observation, *Otobius lagophilus* has only 1 nymphal stage in its life cycle. Two molts occur: the larva to nymph and nymph to adult. The first molt and the transformation to a nymph is completed *in situ* during the engorgement period. The adults do not feed and the tick completes the parasitic phase of the life cycle on 1 host.

Cooley and Kohls (1944) employed the technique used by Brumpt (1936) of measuring the length of the hypostome and the relative size and heaviness of the legs of nymphs which had engorged for various lengths of time, and confirmed his finding of 2 nymphal stages in the life cycle of *Otobius megnini,* the only other known species in this genus. Larval ticks which they placed on the ears of rabbits soon engorged. Some of them were removed and held for molting and others were left attached. Fourteen days after infestation they compared the nymphs that resulted from engorged larvae which had been removed from the host and were later designated as the first nymphs, with the nymphs which remained on the rabbit and were designated as the second nymphs. They found the 2 types to be distinctly different and concluded that the nymphs which remained on the host had undergone a molt. The differences (page 27) they cited are as follows: "The hypostome of the first nymph measured 0.195 mm, the coxae were smaller, and the legs not as heavy. The hypostome of the second nymph measured 0.33, the coxae were larger, and the legs heavier."

In the present study the hypostomes of *O. lagophilus* nymphs were measured, and except for a certain group of measurements, their lengths remained within the boundaries of individual variation, indicating the presence of only 1 nymphal stage. There was also no discernible difference in the heaviness of the legs in this species. In the exception mentioned, engorgd larvae were removed for molting after they had spent 10, 11 and 12 days on the host. After molting, the nymphal mouth parts were removed and mounted and the hypostomes were measured. In all but a few instances, the hypostomes of these specimens were shorter than the hypostomes of nymphs which had remained on the host for longer periods of time. The greatest difference, which was 0.159 mm, occurred between specimens 14 and 32, Table III. They had spent 11 and 15 days respectively on the host, and were removed as engorged larvae before molting. On the other hand, specimens 33 and 35 illustrate a close relationship with respect to hypostome length. Both spent approximately the same number of days on the host and molted at about the same time. Number 33 was removed as an engorged larva and molted off the host, and number 35 molted on the host. In this experiment, if an *O. lagophilus* larva was attached to a rabbit for thirteen days, the hypostome of the resultant nymph approached or reached maximum size (Table III). Exceptions to this, however, can be seen in specimens 7, 9, 11 and 19, which had spent only 10 to 12 days on the host. The extent of engorgement rather than the number of days spent on the host is thought to be responsible for these exceptions. Accordingly, the writer believes that within its natural size limits, the length of the nymphal hypostome is governed by the degree of engorgement attained by the larva before it molts. Therefore, it is suggested that major differences in hypostomal lengths of nymphs that have engorged

for varying periods of time as larvae may not mean that a molt has occurred. This, at least, is true for *O. lagophilus*, where direct observation has indicated the presence of only 1 nymphal stage; and may also be true for *O. megnini*.

On many occasions engorged larvae and partially engorged nymphs were found in the fecal tray of the rabbit's cage. During 1 observation period, 2 recently molted unengorged nymphs with larval exoskeletons still hanging from them, were found unattached among the hairs of the rabbit. Apparently the rabbit had dislodged these ticks by scratching. Whether reattachment ever takes place in such instances is not known.

There was wide variation in the time interval between detachment of engorged nymphs and molting to adults. The average time required for 25 nymphs obtained from jack rabbits shot in the field was 14.6 days per tick; 84% of these nymphs molted in 10 to 15 days, the remainder in 16 to 27 days. Another group of 199 nymphs obtained from a similar source molted to adults in 16 to 23 days. Forty-nine nymphs reared in the laboratory required an average of 24.5 days for the molt; 67% molted in 13 to 25 days, and 33% in 26 to 98 days. Another group of 44 laboratory-reared specimens averaged 23.8 days; 45% molted in 14 to 24 days, and 55% in 25 to 32 days.

Collection records of Beck (1955), Cooley and Kohls (1944), Rosasco (unpublished report) and the writer show that specimens have been taken from rabbits during every month of the year. The most heavily infested *L. californicus* encountered by the writer yielded 1,228 nymphal *O. lagophilus* 20 days after it was captured. This rabbit was approximately 10 days old when it was caught on March 21, 1956. Its head harbored the majority of the ticks; however, ticks were also found attached to the dorsal surface of the body as far posterior as the base of the tail. The largest numbers of *O. lagophilus* previously taken from jack rabbits by the writer were 73 and 102 nymphs respectively from 2 animals captured July 14, 1955 at Rush Valley, Utah. These two were the only rabbits found to be infested in 250 examined. In general, infested rabbits yield only from 1 to 40 engorged nymphs, (Beck, 1955, and Cooley and Kohls, 1944).

### *Adult Stage*

Adults of this species have rudimentary mouth parts and do not feed. As nymphs, they have obtained sufficient blood for completion of the cycle.

In this study, measurements were made soon after emergence of adults. The size limits of adult ticks reared on the jack rabbit in the laboratory varied considerably from figures given by Cooley and Kohls (1944). Since size was used by these authors as the first of several criteria for separating *O. lagophilus* from *O. megnini*, measurements were taken to determine the significance of the variations (Table I). The table shows that both male and female *O. lagophilus* exceed

TABLE I.—*Comparative lengths and widths of Otobius lagophilus*

| Sex | Limits given by Cooley and Kohls (1944) | Limits found in this study |
|---|---|---|
| | mm. | mm. |
| Female | Length 5.40–6.25<br>Width 3.60–4.00 | 5.38–8.75<br>3.25–5.88 |
| Male | Length 4.75–5.00<br>Width 2.90–3.50 | 5.88–7.88<br>3.50–4.88 |

the length and width measurements given by Cooley and Kohls (1944). Table II shows by percentage, the size ranges obtained in this study. Of ninety-two specimens measured, 43% were forcibly removed from the rabbit as nymphs; 57% detached naturally. Perhaps complete engorgement upon their natural host was conducive to the larger size limits found in this study.

Adults were sexed soon after molting from the nymphal stage. Each of 29 females was paired with a male; 10 of these were paired the same day they molted, and the remaining 19 within 1 to 12 days thereafter. An average of 14.6 days was required between pairing and the initial appearance of eggs. In 66% of the cases, eggs appeared within 6 to 15 days; in the remainder 16 to 29 days were required.

During copulation the male and female embrace, ventral surfaces together, with their anterior ends apposed. The genital apertures which are antero-ventral in position are thereby brought into close juxtaposition. A spermatophore is then inserted into the female by the male. An actual transfer of this structure from

TABLE II.—*Percentages* of 53 adult females and 39 adult males in various length and width categories*

| | Females | | Males | |
|---|---|---|---|---|
| | % | Size range | % | Size range |
| | | mm. | | mm. |
| Length | 49 | above 8.00 | 3 | 7.56–8.00 |
| | 28 | 7.56–8.00 | 33 | 7.06–7.50 |
| | 15 | 7.06–7.50 | 44 | 6.56–7.00 |
| | 6 | 6.56–7.00 | 18 | 6.00–6.50 |
| | 2 | Less than 6.00 | 3 | Less than 6.00 |
| Width | 60 | above 5.00 | 13 | 4.56–5.00 |
| | 30 | 4.56–5.00 | 69 | 4.06–4.50 |
| | 8 | 4.06–4.50 | 15 | 3.56–4.00 |
| | 2 | 3.00–3.50 | 3 | 3.00–3.50 |

* Nearest whole number.

male to female was not observed, but an observation made immediately after separating a pair of ticks in copulation disclosed a spermatophore protruding from the female genital aperture. Spermatophores are regularly found in the vials within a few days after the ticks are paired.

Copulation of adults was essential to the production of fertile eggs, but in 2 instances, at least, was not necessary for oviposition. One adult unmated female that was removed as an engorged nymph from a cottontail rabbit laid 5 eggs. Another unmated female obtained as an engorged nymph from a jack rabbit laid 4 eggs. None of the unfertilized eggs hatched.

### *Eggs and Larval Stages*

The number of eggs laid by an individual female was noted for only 3 specimens. One laid 207 in 10 days, another 312 in 19 days, and the last 655 in 76 days.

The incubation period for eggs laid by 24 females averaged 17.5 days. In 79% of the cases, hatching took place within 13 to 18 days and in 21% within 19 to 24 days, at room temperature and approximately 81% R. H.

With few exceptions, females and their eggs and larvae were kept together in the same vial. This made it impossible to determine the ages of the larvae the day that they were applied to the host, although first hatching dates in the vials was known. The period from first hatching to time of application to a host varied from

a few to 45 days. In all the experimental infestations of the jack rabbit, some attachment was noted but the percentages were not computed.

On the jack rabbit, the larvae required 14 to 16 days from the time they were applied until they molted to the nymphal stage, although in 1 instance a newly molted nymph was found on the jack rabbit 29 days after application. This may have been due to a delay in the initial attachment. On a white rabbit the earliest molt was on the 17th day.

TABLE III — *Showing hypostome measurements* of larvae and nymphs of* Otobius lagophilus

| No. | Stage Measured | Removed as an Engorged Larva | Removed as a Nymph | Molted | Days Spent on Host | Length of Hypostome |
|---|---|---|---|---|---|---|
| | | | | | | mm. |
| 1. | Larva unengorged | — | — | — | 0 | 0.187 |
| 2. | Larva unengorged | — | — | — | 0 | 0.185 |
| 3. | Larva unengorged | — | — | — | 0 | 0.182 |
| 4. | Larva unengorged | — | — | — | 0 | 0.192 |
| 5. | Larva unengorged | — | — | — | 0 | 0.178 |
| 6. | Larva engorged | Oct. 7, 1955 | — | — | 10 | 0.192 |
| 7. | Nymph | Feb. 27, 1956 | — | Feb. 28, 1956 | 10 | 0.274 |
| 8. | Nymph | Feb. 27, 1956 | — | Mar. 12, 1956 | 10 | 0.228 |
| 9. | Nymph | Feb. 27, 1956 | — | Mar. 12, 1956 | 10 | 0.247 |
| 10. | Nymph | Feb. 27, 1956 | — | Mar. 16, 1956 | 10 | 0.230 |
| 11. | Nymph | Feb. 28, 1956 | — | Mar. 8, 1956 | 11 | 0.238 |
| 12. | Nymph | Feb. 28, 1956 | — | Mar. 13, 1956 | 11 | 0.209 |
| 13. | Nymph | Feb. 28, 1956 | — | Mar. 16, 1956 | 11 | 0.197 |
| 14. | Nymph | Feb. 28, 1956 | — | Mar. 20, 1956 | 11 | 0.175 |
| 15. | Nymph | Feb. 28, 1956 | — | Mar. 19, 1956 | 11 | 0.202 |
| 16. | Nymph | Oct. 7, 1955 | — | Oct. 13, 1955 | 11 | 0.216 |
| 17. | Nymph | Oct. 7, 1955 | — | Oct. 13, 1955 | 11 | 0.214 |
| 18. | Nymph | Oct. 7, 1955 | — | Oct. 13, 1955 | 11 | 0.211 |
| 19. | Nymph | Feb. 29, 1956 | — | Mar. 9, 1956 | 12 | 0.271 |
| 20. | Nymph | Feb. 29, 1956 | — | Mar. 9, 1956 | 12 | 0.230 |
| 21. | Nymph | Feb. 29, 1956 | — | Mar. 20, 1956 | 12 | 0.182 |
| 22. | Nymph | Jan. 23, 1956 | — | — | 13 | 0.252 |
| 23. | Nymph | Jan. 23, 1956 | — | — | 13 | 0.288 |
| 24. | Nymph | Jan. 23, 1956 | — | — | 13 | 0.302 |
| 25. | Nymph | Jan. 24, 1956 | — | — | 14 | 0.302 |
| 26. | Nymph | Jan. 24, 1956 | — | — | 14 | 0.293 |
| 27. | Nymph | Jan. 24, 1956 | — | — | 14 | 0.264 |
| 28. | Nymph | Jan. 24, 1956 | — | — | 14 | 0.293 |
| 29. | Nymph | Jan. 25, 1956 | — | — | 15 | 0.300 |
| 30. | Nymph | Jan. 25, 1956 | — | — | 15 | 0.293 |
| 31. | Nymph | Jan. 25, 1956 | — | — | 15 | 0.288 |
| 32. | Nymph | Jan. 25, 1956 | — | — | 15 | 0.334 |
| 33. | Nymph | Oct. 12, 1955 | — | Oct. 13, 1956 | 16 | 0.264 |
| 34. | Nymph | — | Oct. 12, 1955 | — | 16 | 0.259 |
| 35. | Nymph | — | Oct. 13, 1955 | — | 17 | 0.286 |
| 36. | Nymph | — | Oct. 14, 1955 | — | 18 | 0.252 |
| 37. | Nymph | — | (naturally detached) Nov. 9, 1955 | — | 44 | 0.300 |
| 38. | Nymph | — | (naturally detached) Nov. 14, 1955 | — | 49 | 0.250 |

* Measurements made from base of posthypostomal hairs to tip of hypostome.

Engorged larvae are oval. The average size of 10 specimens which had fed for 10 to 11 days was 2.60 mm in length and 1.64 mm in width. The lengths were measured exclusive of the hypostome. Their legs are often distorted and are apparently functionless. Engorged larvae which were forcibly removed were never observed to walk.

The total number of days between application of larvae to the host and the natural drop of engorged nymphs was variable. Observations were made on 2 separate groups of ticks. The first group of larvae was placed on a jack labbit August 2; the last nymph dropped off September 20. The 78 nymphs that were recovered

remained on the rabbit for an average of 30.4 days; 76% of these dropped from the rabbit within 23 to 30 days; the remainder in 31 to 49 days. In the second group of 82 specimens recovered, an average of 39.3 days was spent on the host. In this group, larvae were placed on the host September 26, and the last engorged nymph dropped on November 21; 87% dropped off in 28 to 38 days, and 13 per cent in 39 to 56 days.

## SUMMARY

This study of the life history of the soft-bodied tick *Otobius lagophilus* has shown that:

1. The life cycle stages comprise egg, larva, nymph and adult.
2. Larvae remain attached to the host while they molt and transform to nymphs; approximately 14 to 17 days on the host is required before the larva molts to nymph. The total number of days from initial experimental application of larvae until the natural drop of engorged nymphs varied from 23 to 56 days.
3. The average number of days between the cessation of feeding of nymphs and their molt to adults varied from 14.6 days to 24.5 days.
4. A period of 6 to 29 days after adults were paired was required for the initial appearance of eggs; the average incubation period was 17.5 days.
5. The length of the hypostome as an indicator for the presence of 2 nymphal stages may not be a valid criterion.
6. The size limits of adult *O. lagophilus* varied considerably from those given by Cooley and Kohls (1944).

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# FLEAS ASSOCIATED WITH NESTS OF THE DESERT WOOD RAT*

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The desert wood rat, *Neotoma lepida lepida* Thomas, is ordinarily associated ecologically with the pigmy juniper forest communities in the Great Basin of Utah. The house of the rodent usually consists of a collection of juniper sticks and other available debris, and is situated at the base of a juniper, above ground in crotches of the juniper, or occasionally in cliffs or bushes. The nest (or nests) is located in the house for protection (Vestal, 1938). The soft, fluffy nest material provides a suitable habitat for many small commensals and ectoparasites. This article is a report of the fleas collected during a year-long study of wood rat nests at Dugway, Utah. The collections were made by Dr. Dorald M. Allred and associates: Ernest J. Roscoe, William J. Bacha, John Smith, Zelda Rottenberg, Mark Rosasco and Dr. Stanley B. Mulaik. Specimens were identified by members of the staff, but those of the genus *Thrassis* were sent to Harold E. Stark and Frank M. Prince, San Francisco Field Station, U. S. Public Health Service, for identification.

The publications of Hubbard (1947) and Holland (1949) have proved useful as background material. This study is the fourth of a series of local investigations into the ecology of the desert wood rat nests. The first three consist of: (1) A study by Beck, Barnum and Moore (1953) which reported on fleas and other consortes found in wood rat nests in parts of Utah, including a few nests from the region near Jericho, Juab County, Utah; (2) Studies by the author (Howell, 1955) in which the host-flea relationships found in the nests in the Jericho area were analyzed; (3) An unpublished study of the ecology of the fauna of wood rat nests in the Cedar Mountains, Dugway, Utah, about 50 miles northeast of Jericho, made by Allred and Roscoe, dealing with the mites taken from these nests. An article by Howell, Allred and Beck, to be published, analyzes seasonal fluctuations of mites found in wood rat nests.

The topography, soil, climate and flora of the Cedar Mountains is discussed by Robinson and Hesson (1954).

## METHODS AND MATERIALS

Methods of collecting nests, extracting arthropods, and preparation of specimens were discussed by Howell (1955). Weather data used in this study were supplied by the Dugway Meteorological Station, which is located 3 to 10 miles from and 300 to 400 feet lower than the elevation of the study area. In this study 1 flea specimen is considered an infestation; 6 or more specimens is reported as a significant infestation.

## RESULTS

From 135 wood rat houses excavated, 160 nests were obtained. Of these, 101 houses (74.8%), and 111 nests (73.7%) were currently in use. Experience in excavating nests soon provided the observers intimate acquaintance with the appearance of houses, enabling them to distinguish the houses which were occupied, prior to excavation. An average of 7.8 fleas was taken from each of the 111 active nests.

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The following is an analysis of the species of fleas collected, with notes regarding their ecology.

CERATOPHYLLIDAE

*Monopsyllus wagneri wagneri* (Baker). This flea is most frequently found in this area on white-footed mice of the species *Peromyscus maniculatus* and *P. truei*. These rodents are known to frequent wood rat houses. The flea specimens collected from wood rat nests are considered to be accidental associates, probably derived from invaders of the rat nest.

*Thrassis bacchi* ssp. This subspecies appears to be an intergrade of *T. bacchi gladiolis* and *T. bacchi caducus* found on the antelope ground squirrel, *Citellus leucurus*, which may live in close association with the wood rat. This may also be an accidental association since only 5 specimens were collected.

*Thrassis aridis campestris* Prince. This species occurs on wood rats, kangaroo rats (*Dipodomys ordii*) and deer mice. Although no record of this species has heretofore been published from this area, 4 specimens were collected from wood rat nests during the course of this study.

HYSTRICHOPSYLLIDAE

*Anomiopsyllus amphibolus* Wagner. This flea is a nest-inhabiting species and is seldom found on its host. Until recently (Beck, *et al* 1953) it was rarely collected and was known only from the type locality (Hubbard, 1947). It was the most abundant species encountered in this study (Table I); 697 specimens were collected.

*Megarthroglossus smiti* Mendez. Prior to the revision of this genus, (Mendez, 1956) *M. smiti* was identified as *M. divisus* ssp. This recently described species was the second most abundant species collected; 111 specimens were taken. Throughout the season of its occurrence, September through January, specimens of this species were found in most of the nests examined.

*Meringis parkeri* Jordan. Twenty-one specimens were collected at irregular intervals. This flea has been found with nearly equal frequency in the nest and on the wood rat. The kangaroo rat is the preferred host of this flea.

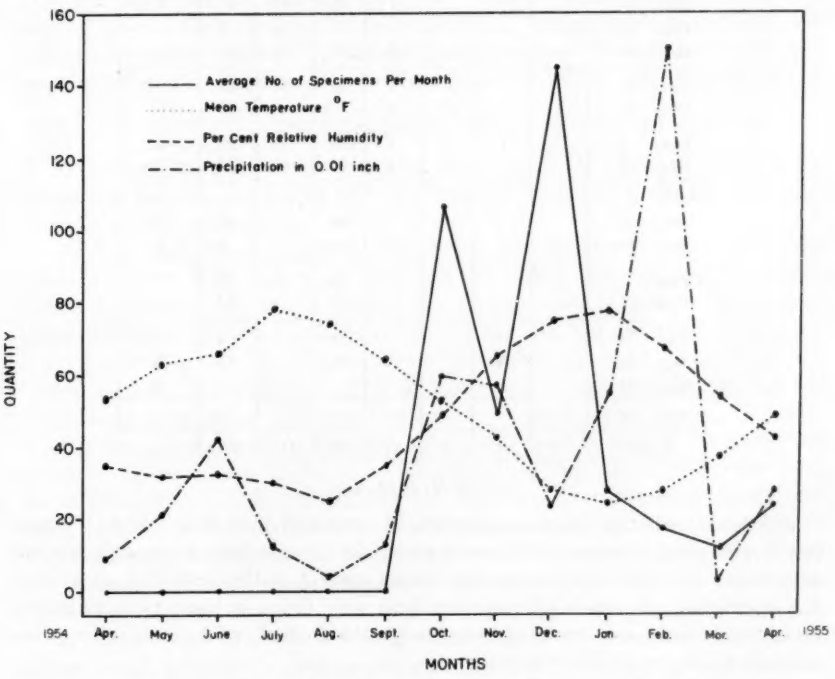


Fig. 1 Seasonal occurence of fleas found in wood rat nests compared with some weather factors

TABLE 1 — *List of comparative figures showing numbers of specimens collected and numbers of nests where specimens were found*

| | FLEAS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Date of Collection | *Monopsyllus wagneri wagneri* | *Thrassis baccbi* ssp? | *Thrassis aridis campestris* | *Meringis parkeri* | *Anomiopsyllus amphibolus* | *Megaribroglossus smiti* | Total number per collection | Number of nests in which species were found |
| 1954 | | | | | | | | |
| April 15 | | | | | | | 0 | 0 |
| April 29 | | | | | | | 0 | 0 |
| May 13 | | | | | | | 0 | 0 |
| May 27 | | | | | | | 0 | 0 |
| June 10 | | | | | | | 0 | 0 |
| June 24 | | | | | | | 0 | 0 |
| July 8 | | | | | | | 0 | 0 |
| July 24 | | | | | | | 0 | 0 |
| Aug. 5 | | | | | | | 0 | 0 |
| Aug. 24 | | | | | | | 0 | 0 |
| Sept. 2 | | | | | | | 0 | 0 |
| Sept. 16 | | | | | | | 0 | 0 |
| Oct. 6 | | | | 1 | 21 | 40 | 62 | 3 |
| Oct. 14 | | | | | 1 | 6 | 7 | 1 |
| Oct. 27 | | | | 2 | 237 | 10 | 249 | 4 |
| Nov. 10 | | | 2 | 1 | 28 | 18 | 49 | 3 |
| Nov. 24 | 1 | | 1 | 8 | 35 | 4 | 49 | 4 |
| Dec. 9 | | 1 | | 6 | 130 | 16 | 153 | 6 |
| Dec. 22 | | | 1 | 1 | 123 | 12 | 137 | 7 |
| 1955 | | | | | | | | |
| Jan. 6 | | | | | 46 | | 46 | 5 |
| Jan. 20 | 2 | | | 2 | 2 | | 6 | 3 |
| Feb. 7 | | | | | 24 | 1 | 25 | 3 |
| Feb. 17 | | | | | 8 | 4 | 12 | 4 |
| Mar. 3 | | | | | 4 | | 4 | 1 |
| Mar. 16 | | 4 | | | 15 | | 19 | 3 |
| Mar. 31 | | | | | | | 0 | 0 |
| April 14 | | | | | 22 | | 22 | 2 |
| Totals | 3 | 5 | 4 | 21 | 696 | 111 | 840 | |

*Multi-Nest Houses*

Of the 111 wood rat houses excavated, 11 contained more than 1 nest; 9 houses had 2 nests; and 2 houses had 3 nests each. In 1 house both nests were infested with fleas. In each of the remaining houses, only 1 of the nests had an average flea infestation. In nearly all instances, fleas were found in those nests judged to be currently inhabited, except during the period from April to September, when no adult fleas were found (Fig. 1).

In 1 house containing 2 nests, both were infested with fleas; however the num-

ber in each was low. Four of the 6 species found in this study were represented in the 2 nests. In only 2 other instances in this study were more than 3 species of fleas represented in a single nest. It is possible that temporary visits of other species of rodents may be responsible for the higher numbers found.

### *Rebuilt Nests*

Each house excavated was marked and then checked every 2 weeks. Often the wood rat reconstructed parts of the house in which it built a new nest. New nests were collected at 2-week intervals for 1 year. Of these rebuilt nests, 19 had been excavated during the winter and 2 during the summer. Thirteen of them were infested with fleas. Six nests were collected 2 weeks following previous collections from the same houses, and no significant numbers of fleas were found. In each of the reconstructed houses, the nest and part of the house had been restored in the 2-week interval. It is estimated that at least 1 month was required for significant reinfestation. Where or how the initial infestation of the rat originated is not known, but it may be expected that fleas on the host drop off during reconstruction, thus infesting the new nest.

### *Comparison of Ground Nests with Tree Nests*

During the year, 11 nests were collected from houses 2 to 6 feet above the ground in crotches of juniper branches. Six nests were collected between October and April, 4 of them being infested with fleas. From the 4 nests collected between May and September, no fleas were collected. Two of the 11 nests were not being used by the rat. Only the 2 predominant species, *M. smiti* and *A. amphibolus*, were collected from these nests. The absence of the flea species considered to be accidental indicates that the nests were not frequented by rodents other than wood rats.

## DISCUSSION

This study has confirmed a previous study (Howell, 1955) in which it was shown that the fleas associated with the nests of the desert wood rat occur only during a specific period of the year.

No fleas were found during the late spring and summer months. The population trends in both studies are very similar, except that the peak population occurred earlier in this survey than in the Jericho study, (Howell, 1955) and a slight difference was noted between the September rise and the April–May decline in the graph (Fig. 2). In both studies the population occurrence covered approximately the same period of time. This similarity lends support to the fact that fleas of the wood rat nests are restricted to a specific seasonal period.

The seasonal incidence of flea infestation in the nests cannot be correlated with the time the rat spends in the nest. A rat was seldom seen outside the house except when driven out by the excavation of its nest. Fresh stores of cuttings were found both in summer and winter. Our observations indicate that the rat ordinarily remains in the nest in the daytime and leaves it to forage at night. Doubtless the fleas, if present, would have opportunity to feed and reproduce at any time of the year.

It was indicated by Holland (1949) that humidity and temperature are critical factors which influence population changes. In this study the mean temperature,

mean relative humidity, and precipitation were plotted against total population (Fig. 1). The mean relative humidity from April to September, 1954, was 8% to 12% lower than from October 1954 to April 1955. When the mean relative humidity dropped below 40% and the mean monthly air temperature was above 50 degrees no adult fleas were found in the nests. When the mean air temperature dropped beolw 50 degrees and the mean monthly relative humidity rose above 40%, fleas began to appear. Because precipitation is sporadic, it is assumed that its direct effect upon the flea population is of little significance, although it may affect the

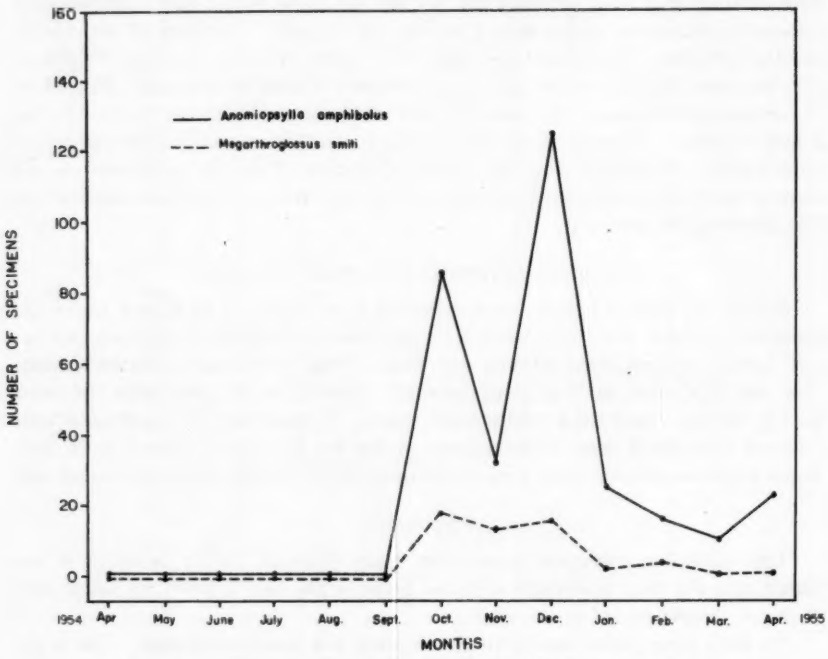


Fig. 2 Seasonal incidence of the most common species of fleas found in wood rat nests

fleas indirectly through changing the temperature and humidity in the nest. Until the microecology of the nest is known and correlated with the macroecology and life cycle of the fleas concerned, the relationship between temperature, humidity and population index cannot be well understood.

In this study, Table II indicates that in the Cedar Mountain area 2 species of fleas predominate in the nests, *Anomiopsyllus amphibolus* and *Megarthroglossus smiti*. These were also more abundant in the study near Jericho. These species were also dominant in the study of Beck, Barnum and Moore (1953), but *M. smiti* was equalled by *Epitedia stanfordi* and surpassed in numbers by *Malaraeus euphorbi*. For comparison, the total numbers of each species taken in the Cedar Mountain and Jericho studies are given in Table III. Despite differences in the numbers of the minor species considered accidental parasites the relative abundance of the major or predominant species is similar in both areas. In this study, and in those studies in the Jericho area, it is noted that there are a number of species of fleas

TABLE II — *A comparison of the species collected at approximately simultaneous periods in each of three studies*

| Species | Jericho Oct. 8 - Nov. 12 1954 | Cedar Mts. Oct. 6 - Nov. 24 1954 | Jericho Oct. 13 - Nov. 27 1952 |
|---|---|---|---|
| | Number of Specimens | | |
| *Malaraeus euphorbi* | 2 | 0 | 45 |
| *Monopsyllus wagneri wagneri* | 0 | 1 | 18 |
| *Orchopeas sexdentatus agilis* | 1 | 0 | 6 |
| *Anomiopsyllus amphibolus* | 270 | 332 | 206 |
| *Atyphloceras echis* | 2 | 0 | 5 |
| *Epitedia wenmanni* | 0 | 0 | 1 |
| *Epitedia stanfordi* | 8 | 0 | 34 |
| *Megarthroglossus smiti* | 97 | 78 | 34 |
| *Meringis parkeri* | 0 | 12 | 1 |

that appear to be accidental parasites (Table III). More accidental parasites were found in the Jericho area than in the Cedar Mountain area. This is probably due to the particular population of associated rodents in the specific locality (Morlan, 1955). If this is true, it would be expected that the degree of accidental host-parasite relationships would be constant from year to year, but only in specific localities. The data from the Jericho area in 1952 and 1954 indicate the degree of host interchange remains constant from year to year (Table III). The present study and the Jericho study both indicate, as suspected, that the host interchange varies from area to area.

Three species of fleas, *Orchopeas leucophus, O. sexdentatus* and *Epitedia stanfordi,* were found in the nests collected in the Jericho area, but were not found in the nests of the wood rat at Dugway although they were of common occurrence on other rodents. Morlan (1955) found a somewhat similar situation existing at the Sante Fe County Airport in New Mexico. The absence of these fleas in the wood rat nests in the Dugway area is probably a result of macroecological factors which are little understood to date. A correlation (Table IV) of the fleas found

TABLE III — *Total number of specimens of this study compared with the total number of the Jerico study*

| Species | Total Specimens Collected | |
|---|---|---|
| | Jericho | Cedar Mts. |
| *Malaraeus euphorbi* | 2 | 0 |
| *Monopsyllus wagneri wagneri* | 11 | 3 |
| *Monopsyllus* sp. | 2 | 0 |
| *Orchopeas leucopus* | 7 | 0 |
| *Orchopeas sexdentatus agilis* | 3 | 0 |
| *Thrassis bacchi* ssp. | 1 | 5 |
| *Thrassis aridis campestris* | 0 | 4 |
| *Anomiopsyllus amphibolus* | 1726 | 697 |
| *Atyphloceras echis* | 27 | 0 |
| *Epitedia stanfordi* | 57 | 0 |
| *Megarthroglossus smiti* | 182 | 111 |
| *Meringis parkeri* | 1 | 21 |

in wood rat nests compared with the same fleas found on other hosts in the study area shows degrees of host interchange. Just where and how this interchange occurred are not presently known.

Three species of fleas, *Epitedia stanfordi, Atyphloceras echis* and *Malaraeus euphorbi,* probably are nest-inhabiting, but may not necessarily be restricted to wood rat nests. Many more rodent nests will have to be collected and examined before the relationship of these species to the rodent will be well understood.

## SUMMARY AND CONCLUSIONS

From a study of 840 specimens of fleas collected from wood rat nests in the Cedar Mountains at Dugway, Tooele County, during a 12-month period from April 1954 to April 1955, it was found that there is a seasonal fluctuation in the flea populations in which adult fleas appear to be absent from April to September, but

TABLE IV — *Correlation chart showing species of hosts from which the fleas found in wood rat nests have been taken*

| FLEAS \ HOST | *Lepus californicus* | *Citellus variegatus* | *Citellus leucurus* | *Eutamias* sp. | *Perognathus formosus* | *Dipodomys ordii* | *Dipodomys microps* | *Reithrodontomys megalotis* | *Peromyscus crinitus* | *Peromyscus maniculatus* | *Peromyscus truei* | *Onychomys leucogaster* | *Neotoma lepida lepida* | *Neotoma cinerea* | *Vulpes macrotis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| • *Monopsyllus wagneri wagneri* | | | | x | | | | x | x | x | x | x | x | | |
| † *Orchopeas leucopus* | | | | | | | | x | x | x | x | | x | | |
| † *Orchopeas sexdentatus agilis* | | | | | | | | | | x | | | x | | |
| • *Thrassis bacchi* ssp. | x | x | x | | | | | | x | x | | x | x | | x |
| • *Thrassis aridis* | | | | | | x | | | | | | | x | | |
| • *Anomiopsyllus amphibolus* | | | | | | | | | x | x | x | | x | x | |
| † *Epitedia stanfordi* | | | | | | | | | | x | | | x | | |
| • *Megarthroglossus smiti* | | | | | | | | | | x | | | x | | |
| • *Meringis parkeri* | x | | | | x | x | x | | | x | | x | x | | |

• Collected from wood rat nests at Jericho.
† Were not collected from wood rat nests at Dugway, but do occur at Dugway.

present during the balance of the year. The most abundant species, *Anomiopsyllus amphibolus* and *Megarthroglossus smiti,* show seasonal peaks in population from October to December. *A. amphibolus* appears in October, reaches its peak population in December, then gradually declines in numbers until it disappears in May. *M. smiti* appears abruptly in October, reaches its peak population density during the same month, and then maintains a relatively stable population until it disappears in May.

A comparison of the multi-nest houses indicates that the rat lives in 1 nest at a time, although other nests may be present in the house and may be used occasionally.

Nests rebuilt during the flea season require at least 1 month to become reinfested with fleas. The mode of reinfestation is unknown.

A comparison between nests from houses built in trees and those built upon the surface of the ground indicates a slight difference in flea population.

The present study shows a correlation between macroecology and population trends, but the explanation for this correlation is not clear. To be understood, the little known microecology should be correlated with the macroecology. Also, the habits of the wood rat may influence the flea population, but all indications are that this is of minor importance.

A comparison of 3 related studies illustrates the variation between populations of the different areas, and within the same area. An understanding of the rodent consortes of the wood rat house would more fully explain the relationship or source of each species of flea within the nest.

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# DISTRIBUTION OF LARVAE OF *TRICHINELLA SPIRALIS* IN THE ORGANS OF EXPERIMENTALLY INFECTED SWINE

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Despite their predilection for development in the voluntary, skeletal muscles of their host, the larvae of *Trichinella spiralis* may also occur in organs that do not contain such striated fibers. The occurrence of trichinae in such organs, either in the preinfective or infective stages of development, has been reported by various workers from 1835 to 1940. The writer (1940) summarized some of these reports and recorded the occurrence of trichinae in the liver, stomach wall, and heart muscle of experimentally infected swine.

Inasmuch as certain of these organs of the pig are used as human food, the ability of trichinae to develop to infectivity in them assumes considerable significance for meat hygiene. Accordingly, the following observations were made between 1940 and 1947 on the distribution and infectivity of trichinae in experimentally infected swine.

## MATERIALS AND METHODS

*Test animals.* Fifty-five pigs of mixed breeds were used. They were 61 days to 8 years old when infected. These pigs were farrowed, housed, and cared for, before and during these tests, at the Agricultural Research Center, Beltsville, Maryland, under conditions which precluded extraneous infections with trichinae.

*Technique of infection.* The pigs were infected by giving to them ground, fresh, or refrigerated pork containing encysted trichinae. The number of parasites per gram of the trichinous pork was estimated by taking small samples from the thoroughly mixed, ground meat until 500 grams had been collected. This meat was digested artificially by the method described later in this paper, and the decapsulated larvae were counted by means of a dilution technique. Although most of the pigs readily ate the infected meat without previous starvation, others did not eat it unless they had been without feed for several hours, or unless the trichinous pork was admixed with the ground feed they were accustomed to eating.

*Technique of examination for trichinae.* The pigs were slaughtered 21 to 162 days after infection. When slaughtered they weighed from 170 to 600 pounds. The organs to be examined for trichinae were removed in the following order: Urinary bladder, liver, gall bladder, intact gastro-intestinal tract with attached pancreas, kidneys, testes or ovaries, uterus and Fallopian tubes, diaphragm, heart, lungs, aorta, and brain. The spinal cord was removed from one pig only. The removal of the diaphragm from each test animal and subsequent treatment to estimate the number of trichinae therein furnished an index of the degree of infection. This determination not only gave assurance that the animal was heavily infected with these parasites but also furnished a quantitative estimate of the varying intensity of infection which might be correlated with the number of larvae recovered from the various organs.

Each organ was carefully dissected out, and its external surface thoroughly washed with tap water from a hose. Prior to the removal of the urinary bladder, the urethra was ligated close to the bladder wall. The urinary and gall bladders were opened and drained, and their interior walls were rinsed into separate sedimentation glasses. Later, the contents of these bladders were examined microscopically for trichinae. The heart, aorta, stomach, and intestines were opened, and their interior walls were washed as previously described. Since in the pig the transition from striated to smooth muscle occurs just anterior to the cardiac portion of the stomach, the forestomach and the diverticulum ventriculi were separated from the remainder of the organ and discarded in order to insure that no striated muscle was included in the portion examined. For the same reason, the part of the liver in contact with the diaphragm was cut off and discarded and each testis was stripped of the tunica vaginalis and the spermatic cord. The brain was removed after carefully opening the skull and exposing the intact meningeal covering. The exposed area was examined for bits of muscle which may have con-

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taminated the area when the skull was opened. After examination, the area was flushed with water, the meningeal covering slit and the brain lifted out.

After removal, processing, and washing, each specimen was placed in a glass jar having a capacity of about 3,000 ml. This jar (plus additional jars if the specimen was large) later served as a container for digesting the specimen. These organs were then ground in an electric food chopper, which was washed in hot water from the tap after each operation. Not more than 1,000 ml of ground material from any one organ was placed in a jar.

The artificial digestive fluid was composed of the following ingredients: water (37° C), 1,000 ml; granular pepsin, 1.5 to 2 grams; and concentrated hydrochloric acid, 10 ml. These components were added to the ground specimen in the order named and were stirred until each was well mixed or dissolved. At least 2 volumes of digestive fluid were added to 1 volume of ground tissue. This mixture was thoroughly stirred, and the container was placed in an incubator (37° C). The suspension was also thoroughly stirred at intervals of 35 to 50 minutes during the first 4 hours of the 18-hour period required for complete digestion.

When digestion was completed, about 2/3rds of the contents of each jar were carefully poured off and discarded. The remaining 1/3rd was poured through a 50-mesh screen into a 5-quart, enameled pot. The screen was flushed with warm water from a hose to remove any enmeshed trichinae. This container was then filled with tap water at 30° C, and the contents allowed to settle for 45 minutes. About 9/10ths of the contents were then poured off and discarded. The remaining 1/10th was poured into a 24-ounce, conical sedimentation glass* and allowed to settle for 45 minutes. All but the lower 3 inches of the contents were then discarded, and the glass refilled with warm water under slight pressure from the tap so that debris which would obscure the larvae would be carried to the surface and could be later decanted. This procedure was repeated at 45-minute intervals until the contents were relatively clear. The supernatant fluid was then decanted, and the sediment was transferred to a flat-bottomed dish and examined under a wide-field, binocular microscope at a magnification of 30 diameters.

All larvae observed were considered viable if they were moving or would move when exposed to water at a temperature of 45° to 50° C. Those that were vacuolated, or had translucent protoplasm and/or a "question-mark" shape, or failed to move on exposure to the warm water were counted as dead. If the viability of the larvae was in question, they were administered by duodenal tube to rats, which were examined for these parasites 30 days later by means of the digestion technique. In some cases, the infectivity of viable trichinae was also tested by using the same procedure.

## RESULTS

The number and distribution of trichinae recovered from the organs of experimentally infected pigs are recorded in Table I.

These data show that all of the pigs became infected with trichinae as was indicated by the recovery of the parasites from the diaphragm. Leaving the spinal cord out of consideration, since only one was examined, the descending order of frequency of invasion of the other organs examined was as follows: Stomach wall, testes, liver, brain, lungs, small intestine, pancreas, aorta, the contents of the urinary bladder, the wall of the urinary bladder, and the heart. There was no correlation between the number of trichinae fed to the hogs and the number found in these locations or between the presence of trichinae in any organ and their occurrence in any other organ. Trichinae were not found in the kidneys, the contents of the gall bladder, the spleen, the ovaries, the wall of the large intestine, or the uterus and Fallopian tubes.

In 7 of the 8 instances in which trichinae recovered from the pigs were administered to rats (see Table I), the test rats became infected. In the one instance in which infection did not occur, only 2 trichinae were fed to the rat. These parasites recovered from the lungs of 1 pig may have been of the same sex. These

* Pilsener-type, thin-walled glasses were used in this operation because they were more efficient and cheaper than similar laboratory glassware. They are available from hotel supply houses.

results indicate that trichinae developing in the stomach wall, liver, brain, lungs, pancreas, and the spinal cord of experimentally infected pigs may attain the infective larval stage.

**TABLE I — *Incidence of infection and number of trichinae recovered from the organs of experimentally infected swine***

| Organ | Pigs Examined (Number) | Pigs Infected (Number) | Pigs Infected (Percent) | Trichinae Recovered (Number) |
|---|---|---|---|---|
| Diaphragm | 55 | 55 | 100 | 1,408* |
| Stomach (wall) | 55 | 10 | 18 | 1; 2; 2; 3; 3; 3; 4; 28**; 55; 2,800 |
| Testes | 20 | 3 | 15 | 1; 9; 1,000 |
| Liver | 55 | 6 | 11 | 1; 2; 8**; 16; 21; 22** |
| Brain | 33 | 3 | 9 | 1; 10; 46** |
| Lungs | 55 | 5 | 9 | 1; 2; 2**; 3; 13** |
| Small intestine (wall) | 11 | 1 | 9 | 2 |
| Pancreas | 42 | 3 | 8 | 1; 43**; 3,520 |
| Aorta | 43 | 3 | 8 | 1; 28; 800 |
| Urinary bladder (contents) | 55 | 4 | 7 | 1; 3; 4; 7 |
| Urinary bladder (wall) | 55 | 3 | 5 | 8; 12; 77 |
| Heart | 55 | 1 | 2 | 1 |
| Spinal cord | 1 | 1 | 100 | 17** |
| Gall bladder (contents) | 55 | 0 | 0 | |
| Kidneys | 55 | 0 | 0 | |
| Large intestine (wall) | 4 | 0 | 0 | |
| Ovaries | 7 | 0 | 0 | |
| Spleen | 48 | 0 | 0 | |
| Uterus and Fallopian tubes | 2 | 0 | 0 | |

* Average number of trichinae per gram of muscle tissue from 26 diaphragms; trichinae from 29 diaphragms not counted. Remaining data from each sample.

** Larvae fed to rats.

## DISCUSSION

The observations reported in this paper confirm the writer's previous findings that under experimental conditions trichinae may occur in organs devoid of striated muscle, and show that such larvae in at least 6 such locations were infective to rats. Since the ability of these larvae to infect a new host was found to be apparently unimpaired, it is considered that the active larvae recovered from other locations, also devoid of striated muscle, probably were infective, although this was not demonstrated by feeding tests.

The finding of trichinae in the contents and wall of the urinary bladder in 2 pigs raised the question as to the manner in which the parasites had arrived in the lumen of the organ. Were they liberated from the bladder wall at the time it was cut or had they entered the bladder by way of the kidneys and ureters? Although it was not possible to answer this question completely, recovery of trichinae from the contents of the bladders of 2 pigs and not from their respective bladder walls, and the recovery of a relatively large number of these worms (77) from the bladder wall of 1 pig and none from the bladder contents, indicated that the parasites in the lumen probably entered the organ by way of the kidneys and ureters.

Leonard and Beahm (1941) examined several thousand sections of the livers, spleens, kidneys, brains, and hearts of experimentally infected rats without finding

trichinae. However, many workers have observed trichinae in various organs containing no striated muscle, as well as in body fluids and in the vascular systems of man and other animals. Whether or not these larvae became encapsulated has never been proved satisfactorily. Thornbury (1897) is the only worker known to the writer who observed and figured encapsulated trichinae in tissues other than striated muscle; these larvae were located in adipose tissue of swine. This author also observed that unencapsulated trichinae were occasionally present even after ample time for encapsulation had elapsed. These findings and those reported in the present paper lend support to the writer's previous recommendation that all organs and tissues of hogs, whether or not they contain striated muscle, should be processed to kill trichinae which may be present, before being used for human food.

The question of encapsulation was not solved by the present investigation because the digestion technique used destroyed many capsules which may have been present. Microscopic examination of the tissues for larvae was not considered feasible in view of the enormous expenditure of time it would require.

## SUMMARY

Fifty-five pigs from 61 days to 8 years of age were experimentally infected, at the rate of 400 to 600 larvae per pound of body weight, with trichinae contained in ground pork.

These pigs were sacrificed 21 to 162 days after infection, and the various organs and tissues were ground in a meat chopper and artificially digested to recover the parasites.

In those instances in which the same organ was examined in more than 1 pig, trichinae were recovered from the following locations, with percentage frequencies given in parentheses: Diaphragm (100), stomach wall (18), testes (15), liver (11), brain, lung, and wall of the small intestine (9 each), pancreas and aorta (8 each), urinary bladder contents (7), urinary bladder wall (5), and heart (2). Trichinae were recovered from the one spinal cord examined, but were not recovered from the kidneys, gall bladder contents, spleen, ovaries, wall of the large intestine, uterus, or Fallopian tubes. Larvae recovered from the stomach wall, liver, brain, lungs, pancreas, and spinal cord were infective to rats. These findings emphasize the importance of cooking or otherwise processing all edible portions of the hog carcass whether or not they contain striated muscle tissue, in order to destroy trichinae.

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# TWO NORTH AFRICAN *IXODES* TICKS: *I. KAISERI* SP. NOV. FROM EGYPTIAN DESERT FOX CUBS. A REDESCRIPTION OF THE FEMALE AND A DESCRIPTION OF THE MALE OF *I. FESTAI* RONDELLI, 1926 (IXODOIDEA, IXODIDAE)

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*Ixodes* ticks are extremely rare and localized in most of North Africa. The discovery of a new species in Egypt, described herein, raises to 2 the species known from that country. As is true for the other Egyptian representative of this genus, *I. redikorzevi redikorzevi* Olenev, 1927 (Arthur, 1955a) the present new species appears to be represented by only small populations in limited geographical areas of Egypt. Other species that appear in North Africa to the west of Egypt are *I. festai* Rondelli, 1926, *I. hexagonus* Leach, 1815, *I. ricinus* (Linnaeus, 1758) and *I. vespertilionis* Koch, 1844.

The female of *I. festai* is redescribed herein in order to enhance our knowledge of the relationships and differences in this genus of ticks and the male of this species is described for the first time.

*Ixodes kaiseri* new species
(Figures 1 to 10)
Egyptian Fox Cub Russet Tick

*Holotype:* Female, well engorged, from immature Common Egyptian Fox, *Vulpes vulpes aegyptiaca* (Sonnini, 1816), Burg El Arab, Mariut, Western Desert Governorate, Egypt, 6 May 1955, H. Hoogstraal *legit.* Deposited in the United States National Museum.

*Paratypes:* Total 9 females. Four engorged females with same data as holotype. Five females from 2 immature *Vulpes v. aegyptiaca* at the same locality as holotype, on 29 May 1956, H. Hoogstraal, M. N. Kaiser and R. Hemsted. Paratypes deposited in collections of the British Museum (Natural History), Rocky Mountain Laboratory, Chicago Natural History Museum, H. Hoogstraal, and the author.

Description of Female:

Body when engorged elongate oval, when partly engorged subcircular. Hairs on dorsum of alloscutum fine, stouter and more strongly curved laterally. Color of sclerotized parts in alcohol-preserved specimens brown.

*Capitulum* (Figs. 1 and 2). Basis capituli very broad, 2/3 as long as broad, breadth across dorsal ridge, 0.48 mm; posterior margin either straight or slightly undulate, lacking cornua, posterolateral angles rounded; lateral margins straight, parallel; surface gently convex anterior of article I, laterally distinctly elevated (Stippled in Fig. 1) and heavily pigmented peripherally; longitudinally depressed between porose areas. Porose areas subcircular but slightly variable, strongly depressed, margins appearing distinctly elevated. Palpi short, broad, with a laterobasal indentation, lateral margin thence straight to apex, mesial surface convex; surface somewhat rugose; hairs long mesodorsally on article II, shorter proximally on article III becoming longer towards apex; length of article II, 0.26 mm, length of article III, 0.21 mm, breadth of article II at junction with article III, 0.18 mm. Ventrally basis capituli very gently curved, lateral margins divergent anteriorly, posterior margin slightly convex, auriculae lacking; margins of basis steep (black in Fig. 2).

*Hypostome* (Fig. 3) terminating anteriorly level with apex of palpi; short, broad, lateral

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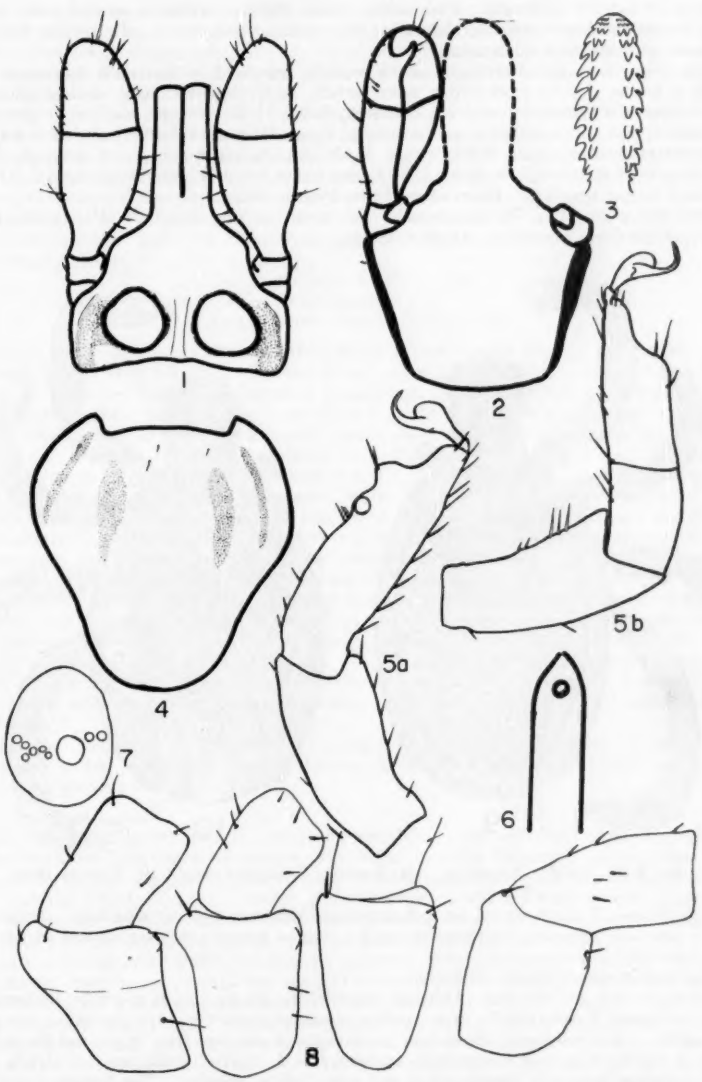



FIGURES 1–8. *Ixodes kaiseri*, sp. nov. Female: 1, Capitulum, dorsal. 2, Capitulum, ventral. 3, Hypostome. 4, Scutum. 5a, Tarsus and metatarsus I. 5b, Tarsus and metatarsus IV. 6, Anal grooves. 7, Spiracular plate. 8, Coxae I–IV.

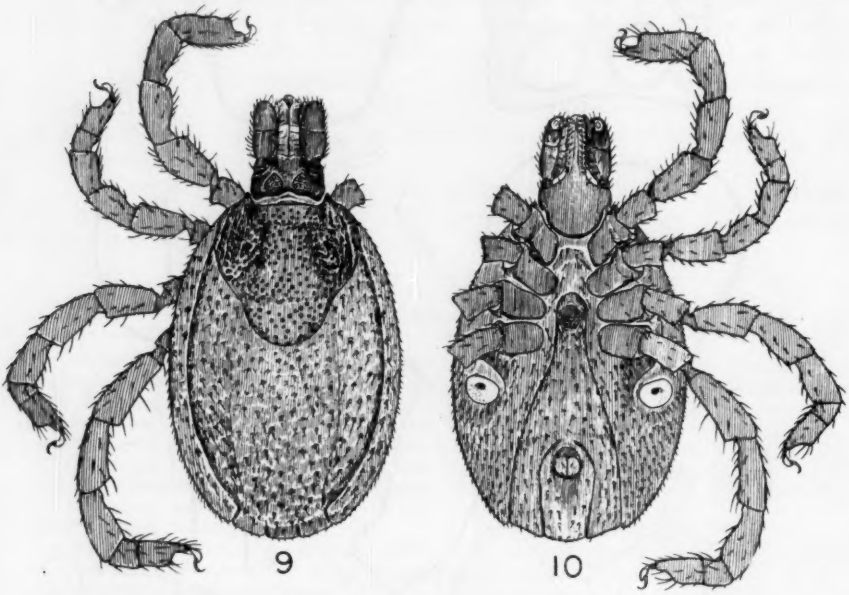
margins parallel; apex rounded; lateral teeth hooklike, dentition 2 files of 4/4, 2 files of 3/3 and 7 files of 2/2; length, 0.38 mm.

*Scutum* (Fig. 4) measuring 1.15 mm in length, 1.14 mm at widest point of anterior third; very broad across anterior third; thence narrowing appreciably and terminating in a broadly rounded margin; scapulae strong, pointed, well separated, emargination well-defined. Lateral carinae slightly rounded elevations, bordered mesially by a shallow longitudinal depression, lateral field frequently rugose. Cervical grooves indicated by broad shallow depressions ex-

tending to or beyond midlength. Punctations small, shallow, widely separated over most of surface, larger anteriorly and anterolaterally; few confluent and deeper. Hairs few, fine, short (compared with those of alloscutum).

*Legs* (Fig. 8). Coxae I strongly convex mesially separated by transverse depression (indicated by a line in Fig. 8) from flatter outer surface, posterointernal angle rounded with small external tuberosity, posterointernal angle salient; coxae II also convex but not so pronounced as in coxae I, also with small external tuberosity; coxae III and IV with small but nevertheless distinct external tuberosities. Tarsi (Figs. 5a, b) all sharply humped and generally broad; length of tarsus I, 0.63 mm, metatarsus I, 0.51 mm; tarsus IV, 0.64 mm, metatarsus IV, 0.52 mm. Claw much longer than pad. Hairs of moderate length, strong.

*Spiracular plate* (Fig. 7), transversely oval, about as long as coxae IV; macula anteroventral, goblets fairly numerous; length 0.32 mm.




FIGURES 9–10. *Ixodes kaiseri*, sp. nov. Female: 9, Dorsal view. 10, Ventral view.

*Anal groove* (Fig. 6) ogival but not appearing definitely to meet anteriorly, straight posterior of anus and extending to posterior margin, region between grooves narrow in newly attached females.

*Male and immature stages:* Unknown.

Related species and remarks: Although superficially *Ixodes kaiseri* might be confused with *Ixodes hexagonus* Leach (1815), it is readily separated from the latter by characters of the basis capituli. In *I. hexagonus* the cornua are variable in size but never large, and the posterior margin of the basis capituli is markedly undulate; in *I. kaiseri* cornua are completely absent and the posterior margin is straight or at best only slightly undulate. The breadth-length ratio of the basis capituli in *I. hexagonus* is as 1.0: 1.18 relative to a comparable ratio of 1.0: 1.43 in *I. kaiseri*, i.e., in the former it is broader and shorter. Palpal article II of *I. hexagonus* is approximately 1½ times the length of article III (1.45: 1.0); in *I. kaiseri* article II is about 1.2 times the length of article III. The breadth of the palp, too, is consistently greater in *I. hexagonus*. Porose areas in *I. hexagonus* are superficial and transversely oval; in *I. kaiseri* they are strongly depressed and either circular or subtriangular.

Of the other known African species, *I. kaiseri* agrees with *I. festai* Rondelli, 1926, *I. cavipalpus* Nuttall and Warburton, 1908, *I. rubicundus* Neumann, 1904, *I. vespertilionis* Koch, 1844, *I. simplex simplex* Neumann, 1908 and *I. s. africanus* Arthur, 1956, in having parallel or slightly divergent anal grooves posterior of the anus. *I. vespertilionis* and *I. s. simplex* are widely ranging species (Arthur, 1956) and are probably widely dispersed during bat flight. Of the 42

species now known to occur south of the southern fringe of the Sahara (Arthur, in preparation), 40 possess either horseshoe-shaped or circular anal grooves. On the other hand, the Palaearctic *Ixodes* except for *I. hoogstraali* (Arthur, 1955b) have divergent or parallel-sided grooves. In other respects *I. kaiseri* does not appear to have affinities with any known species, other than *I. hexagonus*, from this continent.

*I. kaiseri* is represented by only small populations of females in limited geographical areas of Egypt. Attempts to rear this species at NAMRU 3, Cairo, have thus far been unsuccessful.

The species is named for Mr. Makram Nasri Kaiser, Chief Technician of the Department of Medical Zoology, United States Naval Medical Research Unit No. 3, Cairo, Egypt, whose diligent efforts have contributed much of the material and data in this Department.

Hosts: Cubs of the Common Egyptian Fox, *Vulpes vulpes aegyptiaca* (Sonnini, 1816). In spite of considerable search by Hoogstraal and his staff, adults of this species have not been found on adult foxes.

*Ixodes festai* Rondelli, 1926
(Figures 11 to 29)
Festa's North African Russet Tick

*Ixodes festai* Rondelli (1926), pp. 47 to 49, described from 1 female; Figure 1 of scutum, capitulum, anal groove, spiracular plate and tarsus; collected at Derna, Cyrenaica, from *Alectoris barbata* by Professor Festa. Deposition of type not stated. Colas-Belcour and Rageau (1951) p. 364, state that the hypostome is long, dentition 3/3 to 2/2; palps of average size with segment 2 longer than segment 3, which has a rounded apex; material from *"perdix"* (see "Hosts" below).

Original description (freely translated from Italian): "Male, unknown. Female body rounded posteriorly, rather flattened; length 6 mm, width 3.5 mm. Shield slightly longer than broad (1.2×1.1 mm), dark red, with some white hairs, cervical grooves superficial, lateral furrow marked by a depression reaching margin of shield at about two-thirds its length; punctations numerous, small, unequal. Abdomen less heavily pigmented, hairs as on scutum. Vulva between third pair of coxae. Anal grooves united in an ogive anteriorly, parallel posteriorly. Anal aperture small. Spiracular plate at about half body length, transversely oval, white with macula almost in center. Capitulum with base twice as broad as long, lateral margins divergent; posterior lateral angles (cornua) prolonged to a point; porose areas longitudinally piriform, very near posterior margin. Auriculae forming a spine posteriorly and towards the midline (=horn?). Chelicerae, hypostome and palps (?). Legs small at anterior third of body. Coxa I with internal and external spurs, former twice as long as latter (much more so in Fig. 1b), coxae II–IV with small spurs posteroexternally. Tarsi long, humped but not particularly narrow distally."

*Material examined.* Thirty-three fully engorged females from rabbits in Nefifik forest, Morocco, from Dr. J. Brunneau, Institut Pasteur du Maroc, Casablanca; 6 males and 5 females reared from above females.

Redescription of Female.

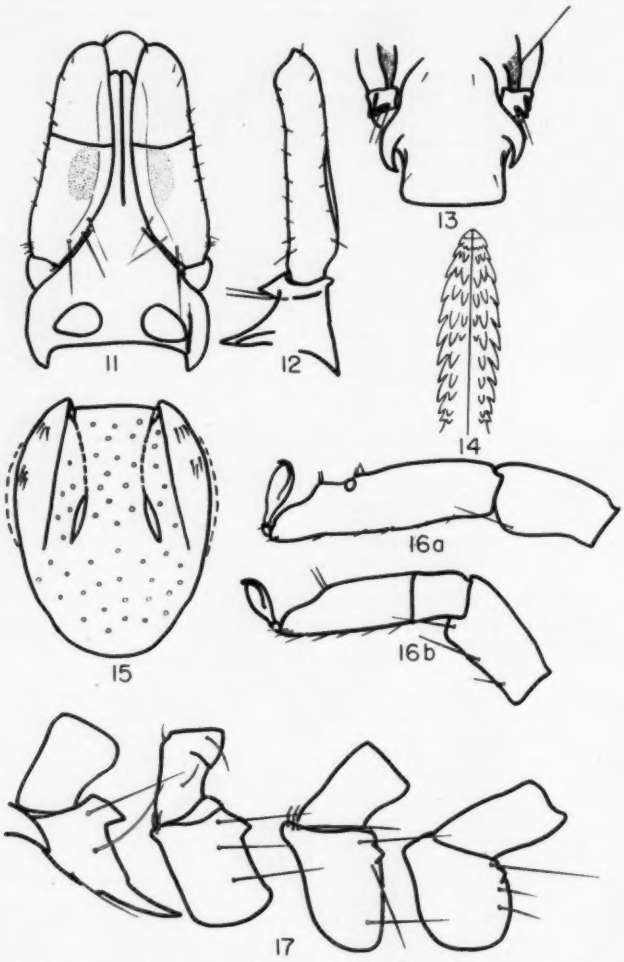
Body broadest behind spiracle, narrowing anteriorly when unfed; elongate oval when fed. Scutum with fine long hairs, alloscutum with dense covering of longer spinose hairs. Color in alcohol preserved specimens reddish-brown to black, particularly the sclerotized parts.

*Capitulum* (Figs. 11, 12, 13) almost black in color; length 0.7 mm, breadth across dorsal ridge 0.4 mm. Basis capituli broader than long; posterior margin straight; cornua strong, longer than basal breadth, narrowing apically; lateral margins broadly rounded or straight, diverging anteriorly. Porose areas small, transversely piriform, separated by wide interval. Region between lateral margins and porose areas elevated and continuous posteriorly with inner edge of cornua. Palpi broad and of moderate length; lateral margin of articles II and III approximately straight; apex rounded; mesial profile gently convex; length of article II, 0.28 mm, article III, 0.23 mm; breadth 0.15 mm. Upper and inner surface of palpi flanged beyond which there is a shallow depression (stippled in Fig. 11). Article I broader than long, giving rise mesodorsally to a short blunt spur. Hairs short laterally, long mesodorsally and proximally. Ventrally (Figs. 12, 13) basis capituli with posterior margin straight, posterolateral angles sharply rounded, leading to a slight waist; auriculae forming pointed, curved horns, extending anteriorly to hypostome by sinuous margins; hairs in two pairs in position indicated in Fig. 13; palpal article I ventrally extended as a strong, pointed spur.

*Hypostome* (Fig. 14) curved in lateral profile, rounded apically; length 0.45 mm; corona small; dentition in 2 files of 4/4, 4 to 5 files of 3/3, 4 to 5 files of 2/2; lateral and mesial teeth pointed and subequal.

*Scutum* (Fig. 15) with surface flattened, almost black in color; longer than broad; length 1.1 mm, breadth 0.84 mm [all available specimens conform to this range (cf. Rondelli, 1.2× 1.1 mm)]; widest at or slightly anterior of midlength; margins gently curved on anterior three-quarters, slightly sinuous before merging with rounded posterior margin, shape variable as de-

picted by broken lines in Figure 15. Lateral carinae distinct, forming straight ridges extending approximately two-thirds of scutal length but not reaching posterolateral margins. Lateral field wide. Cervical grooves arising from inner surface of scapulae as broad, deep, short depressions, becoming faint before passing into short, wide shallow depressions. Punctations large, moderately deep, widely separated. Hairs strong anterolaterally in the lateral field but otherwise fine, long.

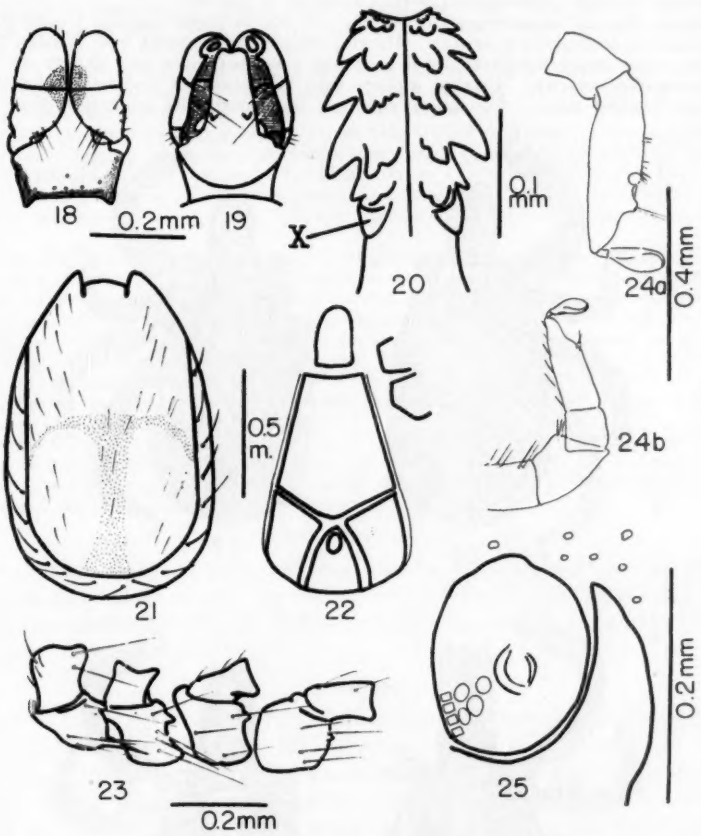



FIGURES 11–17. *Ixodes festai*. Female: 11, Capitulum, dorsal. 12, Capitulum, lateral. 13, Capitulum, ventral. 14, Hypostome. 15, Scutum (broken line indicates variability in outline). 16a, Tarsus and metatarsus I. 16b, Tarsus and metatarsus IV. 17, Coxae and trochanters.

*Legs* (Figs. 16, 17) heavily pigmented, moderate length. Coxa I triangular; internal spur very long, sharply tapering; external spur much shorter. Coxa II with marginal salience posterointernally, external spur smaller than on I. Coxae III and IV with external spurs successively smaller and lacking internal spurs or saliences. All coxae with few, coarse punctations and particularly long, fine hairs. Tarsi short, broad; all humped distally and with apex short and broad; length of tarsus I, 0.54 mm, metatarsus I, 0.30 mm; tarsus IV, 0.44 mm,

metatarsus IV, 0.28 mm; hairs longer and more spinose on tarsus IV than on tarsus I. Pulvillus almost as long as claw.

*Genital aperture* situated level with coxae III. *Anal grooves* in engorged females slightly divergent posterior of anus, appearing ogival anteriorly in fully fed specimens; rounded anteriorly in unfed specimens, arms diverge slightly posteriorly before converging for a short distance and do not reach posterior margin. *Spiracular plate* transversely oval, smaller than coxae IV.




FIGURES 18–25. *Ixodes festai.* Male: 18, Capitulum, dorsal. 19, Capitulum, ventral. 20, Hypostome (X is basal tooth). 21, Scutum and marginal groove. 22, Ventral plates. 23, Coxae and trochanters. 24a, Tarsus and metatarsus I. 24b, Tarsus and metatarsus IV. 25, Spiracle with bordering anterior edge of epimeral plate and pigmented hair plaques.

Description of Male.

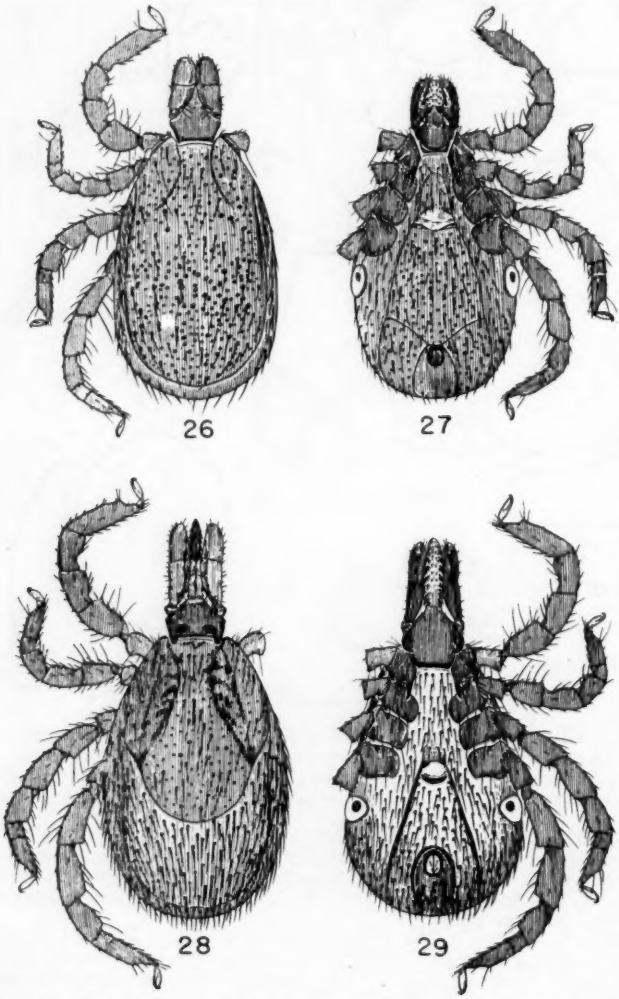
Very small, measuring from 1.43 to 1.46 mm in length, breadth across spiracle, 0.82 to 0.84 mm. Color of legs and capitulum brown, pseudoscutum dark brown, yellow posterior of pseudoscutum except for reddish yellow longitudinal bar and two lateral bars (stippled in Fig. 21). Marginal fold of moderate width.

*Capitulum* (Figs. 18, 19). Basis capituli slightly convex except for a slight depression between palpal bases and hypostome; pores few, scattered. Color dark brown, black-brown peripherally. Posterior margin straight; cornua distinct, triangular; lateral margins straight, divergent to palpal base. Length of basis, 0.11 mm, breadth of basis, 0.2 mm. Palpi short, broad. Article II undulate laterally, mesial profile as a broad arc. Article III with straight lateral profile, mesial profile narrowing slightly to a broadly rounded apex. Length of article

II, 0.11 mm, length of article III, 0.11 mm. Distinct depression mesially and distally on article II, and proximally on article III (stippled in Fig. 18). Hairs short laterally, very long and fine basally on mesodorsal surface of article II. Ventrally transverse ridge crescentic, with a steep slope to hypostomal base, surface posterior of ridge declivitous; posterior margin straight; lateral margins slightly concave, divergent.

*Hypostome* (Fig. 20) approximately 0.2 mm long; 8 sharp lateral teeth; sixth tooth from apex largest; mesial teeth as crenulations indicating a 3/3 arrangement, basal tooth (X in Fig. 20) directed ventrally. Arrangement of teeth as shown in Fig. 20.

*Scutum* (Fig. 21) measuring approximately 1.19 mm in length, greatest breadth 0.7 mm. Surface convex, particularly posteriorly. Margins straight and divergent from scapulae to level of second coxae, thence sharply angled to rectilinear sides that widen very slightly posteriorly, broadly rounded extremity. Cervical grooves short depressions of moderate depth, scapulae long with rounded apices. Punctations uniformly distributed; size moderate; deep. Hairs




FIGURES 26–29. *Ixodes festai*. 26, Male, dorsal view. 27, Male, ventral view. 28, Female, dorsal view. 29, Female, ventral view.

fine, straight; length moderate. Color of pseudoscutum dark brown, posteriorly yellow with bands of reddish yellow markings (stippled in Fig. 21). Marginal fold distinct; hairs strong, curved; longer than those on scutum.

*Ventral plates* (Fig. 22). Pregenital plate extending anteriorly from midlevel of coxae III; posterolateral angles may be either sharply angled or indented; sides parallel; rounded anteriorly. Median plate approximately 1.35 times as long as broad, pentagonal with greatest width posteriorly, anterior margin slightly concave, punctations as on scutum but more widely dispersed, hairs fine of moderate length. Adanal plates broadest anteriorly. Anal plate ogival. Ventral plates separated by wide region of intersegmental membrane. Hairs arise from pigmented plaques.

*Legs* (Fig. 23) short, broad. Coxae with very long, fine, white hairs. Coxa I with very long internal spur; external spur short, rounded apically; coxae II and III with posterointernal saliences; external spur on coxa II approximately equal to that of coxa I, smaller on coxae III and IV. Trochanters lacking spurs. Tarsus (Fig. 24a) broad, short; with sharp hump beyond Haller's organ; narrowing proximally; hump much reduced on tarsus IV (Fig. 24b). Length of tarsus I, 0.38 mm, metatarsus I, 0.16 mm; tarsus IV, 0.28 mm, metatarsus IV, 0.18 mm.

*Spiracular plate* (Fig. 25) elongate, oval, long axis parallel to that of body, outline slightly irregular, length 0.175 mm, breadth 0.139 mm, macula ventral of middle, goblets of moderate size.

Related species: *Ixodes festai* belongs to that group of ticks possessing retrograde auriculae; a very long internal spur on coxa I and shorter external spurs on all coxae with the posterointernal margin of coxa II, at least, drawn out slightly but distinctly to form a marginal salience. In these characters, as well as in that of the deeply punctate scutum, it agrees with the *I. schillingsi* group of ticks. It differs therefrom in the very strongly developed cornua, the possession of a strong ventral spur on article I of the palpi, and in more clublike palpi.

Hosts: Cyrenaican Barbary Partridge, *Alectoris b. barbata* (Reichenow, 1896); *"perdix"* (Colas-Belcour and Rageau, 1951) is probably same species of partridge; rabbits in Morocco (probably *Oryctolagus cuniculus* subsp.).

Remarks: The description of the present material, from Moroccan rabbits, agrees substantially with that presented by Rondelli (1926) except that the scutum is not elongate oval and the punctations are large and of moderate depth. Previously, *Ixodes festai* was known from 2 Libyan and Tunisian specimens from 2 different avian hosts.

ACKNOWLEDGMENTS

Thanks are expressed to Harry Hoogstraal for supplying material and information on *Ixodes kaiseri* sp. nov., for information on hosts of *I. festai*, and for reviewing this manuscript; to Glen M. Kohls for reviewing this manuscript; and to Jean Brunneau for material of *I. festai*.

SUMMARY

*Ixodes kaiseri* sp. nov., widely differing from all known species though more similar to *I. hexagonus* Leach, 1815, than any other, is described from females taken only on weanling cubs of the Common Egyptian Fox on the Mediterranean littoral. It has not been found on adult foxes or other animals, or elsewhere in Egypt. *I. festai* Rondelli, 1926, is redescribed from females and the male is described, for the first time, from reared specimens. Material from Moroccan rabbits (probably *Oryctolagus cuniculus* subsp.) adds to our knowledge of hosts and distribution of *I. festai*, previously known only from Barbary partridges, *Alectoris b. barbata* (Reichenow, 1896), from Libya and Tunisia.

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# *PARAMONOSTOMUM MALERISCHI* N. SP. (TREMATODA: DIGENEA: NOTOCOTYLIDAE) FROM THE EMPEROR GOOSE (*PHILACTE CANAGICA* L.) IN ALASKA

T. T. DUNAGAN*

The trematodes described below were killed by freezing and later fixed in cold AFA. Specimens thus killed are not contracted or otherwise contorted but compare with specimens anesthetized with menthol before being killed. All specimens examined in this study were removed from the cecum of a single emperor goose collected 80 miles east of Nome, Alaska. Measurements represent the range of variation obtained from 15 specimens.

The writer wishes to thank Dr. S. H. Hopkins, Professor of Zoology, Texas A & M College, College Station, Texas, for reading the manuscript and making helpful suggestions.

Class: Trematoda
Subclass: Digenea
Family: Notocotylidae

*Paramonostomum malerischi* n. sp.
(all measurements in millimeters)

Dorso-ventrally flattened body elongate, measuring 4.1–4.3 in egg-producing adults (see Fig. 1); anterior end bluntly pointed with posterior extremity rounded; body uniform in width (0.75–0.79) throughout except for anterior ¼th which tapers slightly toward oral sucker. Studies of whole mounts and serial sections produced no evidence of ventral glands or spination. Integument, however, of entire body surface distinctly marked in a "cross hatch" pattern; transverse lines or minute ridge-like folds may appear as spines when viewed along lateral body margin.

Oral sucker slightly wider than long, measuring 0.135–0.150 long × 0.140–0.150 wide; pharynx lacking; esophagus short and not convoluted. Intestinal ceca simple, anterior portion passing between vitelline gland and uterus and terminal portion passing between testes and ovary, terminating medial to posterior portion of testes.

Testes 0.40–0.60 long × 0.2–0.32 wide, usually deeply lobed on both medial and lateral border; in some instances, however, medial border relatively smooth; lobation varies from specimen to specimen and within same individual, a characteristic common for this group. Vasa efferentia unite anterior to Mehlis' gland to form vas deferens; the latter then proceeds anteriorly along mid dorsal line, gradually increases in size as it approaches the cirrus sac, passing anterior and lateral to base of cirrus sac before entering posterior end of latter structure. Cirrus sac pyriform, 1.90–2.19 long × 0.095–0.150 wide near its base, containing well-developed prostate and unarmed cirrus. Gonopore opens ventral to esophagus and immediately before intestinal bifurcation (see Fig. 3).

Ovary somewhat elongate and slightly lobed, 0.235–0.255 long × 0.180–0.220 wide, located between testes; oviduct leaves cephalic margin of ovary and extends dorsad to Mehlis' gland; a short distance before entering latter, it is joined by common vitelline duct which extends ventrad from union of 2 primary vitelline ducts. Mehlis' gland smaller than ovary and located anterior thereto. Location of oviduct's entrance into Mehlis' gland could not be determined. Likewise, beginning of uterus not determined. Uterus extends anteriad from Mehlis' gland in 12–15 transverse loops before forming metraterm which unites with cirrus sac near posterior border of intestinal bifurcation. Eggs, 0.026–0.028 long × 0.012–0.014 wide, possess long polar filaments (see Fig. 2). Vitelline glands distinct, heavily staining follicles extending from anterior margin of testes to region of cirrus sac. In no case did both bands of vitelline glands reach posterior margin of cirrus sac.

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The specific name was selected from German.
*Host:* Emperor goose (*Philacte canagica* Linn.).
*Location:* cecum.
*Locality:* 80 miles east of Nome, Alaska.
*Specimens:* Paratype, U.S.N.M. Helm. Coll. No. 55254. Type, Arctic Aeromedical Laboratory Helm. Coll. No. 861.

## Discussion

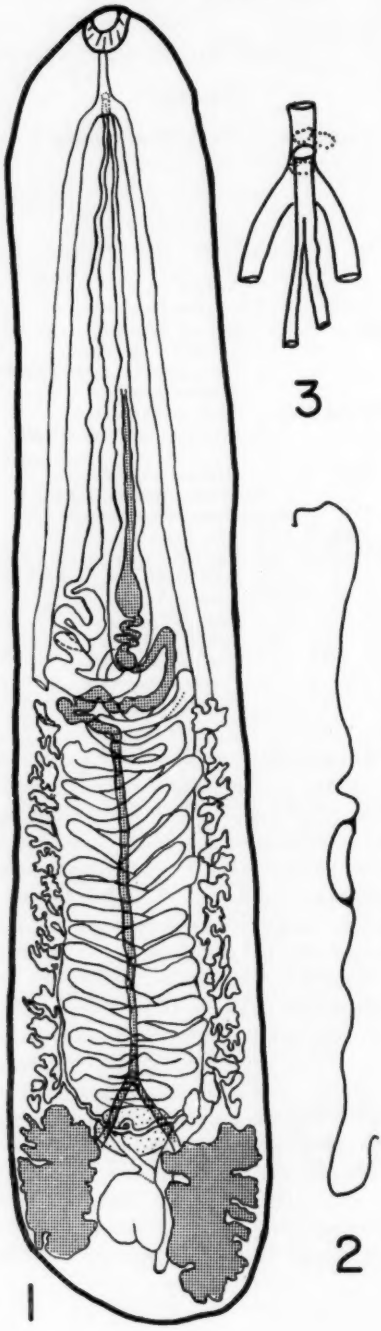
The genus *Paramonostomum* Luehe 1909 is composed of the following species:

| *Parasite* | *Host* | *Locality* |
|---|---|---|
| *P. alveatum* Mehlis 1846 | *Anas penelope*<br>*Branta* sp.; *Somatera* sp.<br>*Anser anser*<br>*Nyroca* sp.<br>*Cygnus cygnus* | Europe |
| *P. ionorne* Travassos 1921 | *Chionis alba*<br>*Iornornis maritinica*<br>*Limnopardalis rythirhynchus* | Brazil |
| *P. echinum* Harrah 1922 | *Ondatra zibethica* | Colorado, USA |
| *P. parvum* Stunkard & Dunihue 1931 | *Chen caerulescens* | Canada |
| | *Glaucionetta c. americana* | Utah, USA |
| | | Long, Island, USA |
| *P. pseudalveatum* Price 1931 | *Ondatra zibethica* | Maryland, USA |
| | *Branta canadensis* (?) | |
| *P. microstomum* Moghe 1932 | *Philomachus pugnax* | India |
| *P. elongatum* Yamaguti 1934 | *Olor bewicki jankowskii* | Korea |
| *P. bucephalae* Yamaguti 1935 | *Bucephala c. clangula* | Japan |
| *P. ovatus* Hsu 1935 | Wild duck (no species) | China |
| *P. casarcum* Lal 1936 | *Casarca ruttla* | India |
| *P. querquedulum* Lal 1936 | *Querquedula circia* | India |
| *P. macrostomum* Ku 1938 | *Fulica atra atra* | China |
| *P. obtortum* Caballero y C 1942 | *Querquedula discors* | Mexico |
| *P. brantae* Bullock 1952 | *Branta canadensis* | New Hampshire, USA |
| *P. malerischi* n. sp. | *Philacte canagica* | Nome, Alaska |

*P. malerischi* can be separated from most of the above species on the basis of the gonopore position. In the former the gonopore is located anterior to the intestinal bifurcation whereas in all but 4 of the above listed species the gonopore is posterior to the intestinal bifurcation. Of the remaining 4 forms, *P. malerischi* can be separated from *P. alveatum* and *P. pseudalveatum* on the basis of uterine loop number. The latter species possess 6–8 uterine loops each whereas *P. malerischi* possess 12–15 uterine loops. *P. casarcum* and *P. querquedulum* have eggs 20 microns or less in length and the cirrus sac is located in the anterior 1/3 of the body. In contrast, *P. malerischi* possesses a cirrus pouch reaching the mid region of the body and anterior uterine eggs 26–28 microns long.

Lal (1936) established the genus *Neoparamonostomum*, which Harwood (1939) pointed out was based on a false assumption concerning the characteristics Lal had selected for his type species. Harwood then synonymized *Neoparamonostomum* with *Paramonostomum* but accepted Lal's idea of separating the genus by tentatively dividing it into 2 groups: *alveatum* and *elongatum*. Neither of these groups, however, possesses characters inherent to one but not to both. The division is, therefore, of little value for systematic purposes. Harwood later stated, "Since there is a possibility that ventral glands may have been overlooked by authors responsible for the second group of species (*elongatum* group), and because of the existence

PLATE I



of the intermediate form, *P. ionorne,* such a step (division of the genus) is at present inadivsable." Because of the limited amount of new information available, this statement is still applicable.

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### Explanation of Figures

Figure 1. Dorsal view of *P. malerischi* with male genital system shaded.

Figure 2. Lateral view of egg showing curvature of the shell and extremely long polar filaments.

Figure 3. Ventral view of gonopore showing variations in location of opening.

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## RESEARCH NOTES

### EXPERIMENTAL TRANSMISSION OF THE DOG HEARTWORM, *DIROFILARIA IMMITIS,* BY *ANOPHELES QUADRIMACULATUS*

The experimental transmission of the dog heartworm, *Dirofilaria immitis,* by subcutaneous injection of infective larvae dissected from infected mosquito hosts has been accomplished on several occasions, recently in Japan by Kume and Itagaki (1955, Brit. Vet. J. **3:** 16–24) and in England by Webber and Hawking (1955, Exper. Parasitol. **4:** 143–164). Recently in our laboratory, in 1955, we injected into a young "clean" dog (Dog 15) an estimated 50 to 75 infective larvae dissected from four *Anopheles quadrimaculatus* which had fed 2 weeks previously on a dog (Dog A) shown at autopsy to be infected with *D. immitis.* Dog 15 had been born and reared in a cement-floored animal room which is washed and treated with insecticides frequently. The occurrence of mosquitoes, or of fleas on dogs maintained in this room, has not been observed. Monthly examinations of ear blood from Dog 15 were made, and microfilariae of *D. immitis* (20 per 20 cu. mm) were first observed in this dog the 7th month after injection. Its blood is still positive for microfilariae 19 months after the innoculation.

However, transmission of *D. immitis* experimentally from dog to dog exclusively by the *bite* of an infected intermediate host does not appear to have been accomplished to any extent. Only 1 report has been encountered, *i.e.,* that of Kume and Itagaki in Japan (1955). They allowed infected mosquitoes (*Aedes togoi*) to feed on recipient dogs, and later recovered filarids from these dogs. While most of the latter had also received subcutaneous injections of infective larvae, according to 1 of their tables, 2 dogs which were found at autopsy to contain heartworms had apparently been exposed to mosquito bites only.

In the fall of 1956, we exposed another dog (Dog 18) born and reared in the animal room mentioned above, to the bites of a total of 55 infected *A. quadrimaculatus* from 2 lots, at 5 different occasions over a period of 3 weeks. These mosquitoes had previously been allowed to feed on Dog 15 which, at the time, had a microfilaremia of 150 per 20 cu. mm during the day. During exposure to Dog 18, the mosquitoes appeared obviously affected by the infection, and apparently had difficulty in feeding. Only 8 were observed to do so; dissections of these and of some which did not feed indicated rather heavy infections. One of the latter had as many as 27 infective larvae in its head and proboscis.

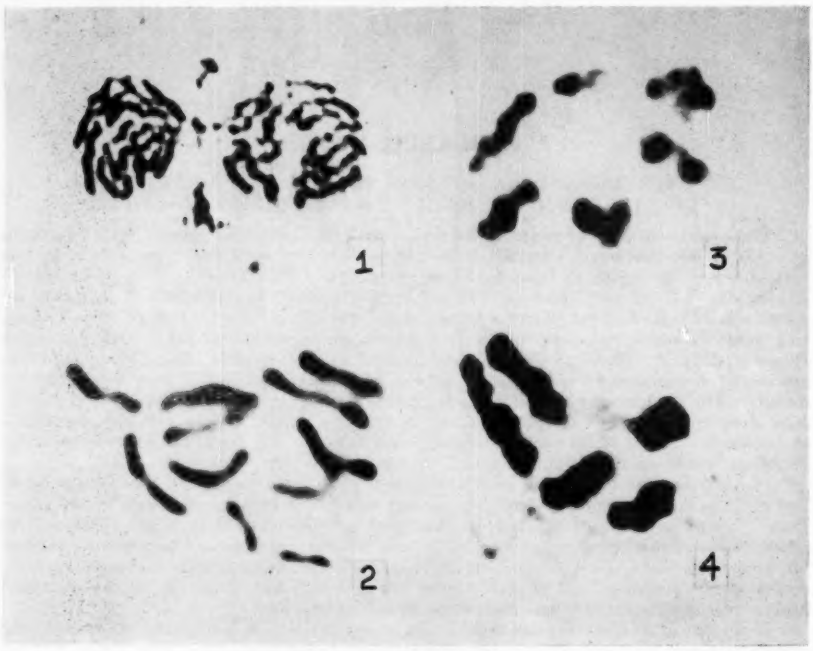
Monthly examinations of ear blood from Dog 18 were made, and through the 6th month after exposure the dog was negative. Unfortunately, the dog was not examined during the 7th and 8th month. Nine months after infected *A. quadrimaculatus* had fed on this dog, the first blood smear containing microfilariae diagnosed as *D. immitis* was obtained. The count was low, only 3 microfilariae per 20 cu. mm of blood. At 10 months, the count has risen to 20 per 20 cu. mm.—Walter L. Newton (with the technical assistance of Paul C. Shade), *Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 14, Maryland.*

## THE CHROMOSOMES OF *STEPHANURUS DENTATUS* (NEMATODA: STRONGYLOIDEA)

A recent paper by Bremner (1955, Austral. J. Zool. **3**: 312–323) has directed attention toward the value of chromosome morphology as an aid in nematode taxonomy. Recently the writers had occasion to collect large numbers of adult kidney worms. Although the genus *Stephanurus* contains only this species, *S. dentatus*, the availability of abundant material provided an opportunity to attempt to determine the chromosome number and morphology for this genotype and for the practice of techniques which might later be applied to other genera with several species.

Kidney worms were obtained from the Armour Company packing plant in Tifton, Georgia. Upon arrival at the laboratory the nematodes were dissected from the tissues, washed in 0.8% saline, and stored at 10° C until needed. Gonial tissue was removed from the worms by the technique outlined by Bremner. Temporary squash preparations with acetic orcein were made from these tissues in the usual manner. Most of the preparations were not suitable for a study of chromosome morphology; however, some were satisfactory for chromosome counting. Accordingly, the number of chromosomes, not heretofore determined, is reported herein.

The diploid number in females was found to be 12. Figure 1 shows a late prophase




FIGURES 1–4. The chromosomes of *Stephanurus dentatus*, showing respectively, oogonial prophase ×3000, spermatogonial metaphase ×6500, spermatocyte metaphase ×4500, and spermatocyte metaphase ×5600.

in 2 oogonia from the germinal tip of the ovary. The diploid number in the male is 11 (Fig. 2). Acceptable preparations of oocytes showing the haploid chromosome complement were not found; many figures of spermatocytes were obtained from the mid-region of the testes (Figs. 3 and 4). All chromosomes figured are from gonial tissue stored in the refrigerator for no longer than 2 days.

Preparations made from worms stored in the refrigerator for up to 6 days were satisfactory for chromosome enumeration. However, as the period of storage was increased up to this limit, shortening and thickening of the chromosomes, particularly those of the female worm, occurred; this conclusion was based on examination of preparations of cells always taken from the identical region of the gonad.—FRANCIS G. TROMBA AND ARNOLD E. STEELE, *Agricultural Research Service, U. S. Department of Agriculture, Animal Disease and Parasite Research Division, Tifton, Georgia.*

## ON THE HOMONYMY OF *EIMERIA FULVA* FARR, 1953 AND *EIMERIA FULVA* SEIDEL, 1954

In 1954, Sprehn described *Eimeria* (*Globidium*) *perniciosa* from the nutria, *Myocastor coypus*. The considerable size (48–50 microns) of the oocysts as well as the thickness and dark brown color of the cyst wall of this species seemed to correspond to the characters given by several authors for the other species of the doubtful *Globidium* group. Seidel (1954) realizing that the name was preoccupied by *Eimeria perniciosa* (Allen, 1936) Neveu-Lemaire, 1943 (= *Tyzzeria perniciosa* Allen, 1936) renamed this coccidium *Eimeria* (*Globidium*) *fulva*.

The *Globidium* group [according to Henry and Masson (1932) "genus", in Reichenow's (1953) opinion "subgenus"] was assigned in the *Catalogue of the genus Eimeria* (Pellerdy, 1956, Acta Vet. Acad. Sci. Hung. **6**: 75–102), without any taxonomic distinction, to the genus *Eimeria*. This was necessary since, as I pointed out in my catalogue, *Globidium* is a synonym of *Eimeria*. That is why I altered the names of some *Eimeria* species. Thus, for instance, I used the name *Eimeria fulva* for the coccidium of nutria, in place of Seidel's denomination *Eimeria* (*Globidium*) *fulva*.

Recently I learned that the name *Eimeria fulva* Seidel, 1954 was preoccupied by *E. fulva* Farr, 1953. According to the Rules of Zoological Nomenclature, the prior name is to be preserved. Thus Seidel's later denomination for the species from the nutria must be changed.

I propose the new name *Eimeria seideli* for the species found by Sprehn in the nutria. Accordingly, some corrections become due in my above mentioned catalogue:

1. *Eimeria fulva* Farr, 1953
   Homonym: *Eimeria fulva* Seidel, 1954 in *Myocastor coypus* (See: *E. seideli*).
   25.6–32.4 × 20.2–25.2 microns
   Hosts: *Branta canadensis canadensis, Anser anser anser.*
Aves. Anseriformes.
2. *Eimeria seideli* nom. nov.
   Synonyms: *Eimeria* (*Globidium*) *perniciosa* Sprehn, 1954; *Eimeria* (*Globidium*) *fulva* Seidel, 1954. Non: *Eimeria fulva* Farr, 1953
   48–50 × 42 microns
   Host: *Myocastor coypus.* Rodentia. Octodontidae.—L. PELLÉRDY, *Department of Parasitology, Veterinary Research Institute, Hungarian Academy of Sciences, Budapest, Hungary.*

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